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NAME OF AUTHOR/*NOM DE L'AUTEUR* Colwell, Douglas D.

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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. J. L. Mahrt

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ASPECTS OF THE LIFE CYCLE, DEVELOPMENT AND ULTRASTRUCTURE OF SARCOCYSTIS (PROTOZOA: SPOROZOA) FROM MOOSE (ALCES ALCES)

by

© DOUGLAS D. COLWELL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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EDMONTON, ALBERTA

FALL, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Aspects of the Life Cycle, Development and Ultrastructure of _Sarcocystis_ (Protozoa: Sporozoa) from Moose (_Alces alces_)" submitted by Douglas D. Colwell in partial fulfilment of the requirements for the degree of Master of Science

J. Mahrt
Supervisor

P.H.G. Stockdale

J. Holmes

L. Wang

Date Sept. 12, 1977

ABSTRACT

Beagle pups fed Sarcocystis (Protozoa:Sporozoa) infected moose (Alces alces) muscle shed characteristic sporocysts 15.6 um (14.5-15.8) x 11.4 um (10.8-11.5) in their feces 11-15 days after infection. Cats and one coyote which also received sarcocyst infected moose muscle passed no sporocysts.

Histological examination of intestinal tissue from experimatally infected dogs revealed microgametocytes, macrogametes and oocysts. These developmental stages were present in the lamina propria of the small intestine and were usually in the luminal third of the villi. Developmental stages were concentrated in the proximal half of the small intestine. Oocysts were first found in tissues 7 days post-infection and a sequence of sporogonic development was noted on subsequent days.

Ultrastructural examination of endogenous stages was limited. However, details of wall forming bodies, oocyst wall and sporocyst wall were noted. Wall forming bodies measured 0.31-0.55 nm in diameter, the complete oocyst wall was thin (89-13 nm) and was often not present around partially sporulated sporoblasts. Sporocyst wall formation occurred in a manner similar to that known for oocyst wall formation and resulted in a bilayered wall 89-155 nm in diameter. Tubular inclusions 67nm in diameter extended from the parasitophorous vacuole membrane into the vacuole.

Thirty-two of forty moose examined were infected with Sarcocystis. Two types of gross cyst structure were noted; one long and fusiform (Type I), and the other spherical to oval (Type II). These were difficult to distinguish in parrafin sections, but were found to have distinct ultrastructural features. On the basis of cyst ultrastructure and the

completion of one life cycle it is suggested that the two cyst types represent undescribed species of Sarcocystis.

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CHAPTER I INTRODUCTION

Members of the genus Sarcocystis are intracellular parasitic protozoa (subphylum Apicomplexa, class Sporozoa, family Eimeriidae) whose characteristic white cysts were first described from the skeletal muscles of mice by Miescher in 1843 (Scott 1943). Cysts have since been reported from striated and/or cardiac muscles of organisms representing all classes of vertebrates except the Amphibia (see reviews by Levine 1973 and Drouin 1976).

The protozoan affinities of Sarcocystis have been doubted on occasion. Spindler and Zimmerman (1945) reported sarcocysts found in swine to be the fungus Aspergillus sp. These authors also reported the appearance of muscle cysts in swine inoculated with "fungal" material cultured from the original infection. There are also reports of "the fungus Sarcocystis" in parasite surveys of moose in North America (Devos & Allin 1949, Peterson 1955). All doubts were laid to rest when electron microscope studies on cysts and their contents by Ludvik (1956, 1958, 1960), Zeve et al. (1966) and Senaud (1967) elucidated a cyst morphology which bore a remarkable resemblance to that known for cyst forming Sporozoa (e.g. Toxoplasma, Besnoitia and Frenkelia). The same studies revealed organelles and inclusions within cystozoites (=merozoites) which are characteristic of motile sporozoan sporozoites and merozoites (i.e., conoid, rhoptries, micronemes, pellicular complex).

Early attempts to establish the life cycle of Sarcocystis were conducted under the assumption that the life cycle was direct (i.e., the parasite was transmitted from host to host without a period of development in an intermediate host). Smith (1901, 1905), Negre (1907), Darling (1910a,b) and McGowan (1923) reported the successful direct transmission of *Sarcocystis*. In retrospect it appears that these workers either dealt with mixed infections of *Sarcocystis* and *Toxoplasma* (which is known to be capable of direct transmission) and failed to distinguish between the two, or their experimental design did not preclude contaminative infection of the experimental hosts.

In two notable cases completion of the life cycle was missed either through a chance decision or through failure to recognize the implication of the experimental results. Scott (1915) placed uninfected lambs in an enclosure which had been previously occupied by dogs fed sarcocyst-infected sheep muscle. He reported no infection in the lambs, but on the basis of present knowledge it is likely that the dogs were removed from the enclosure before infective sporocysts would have been present in their feces; thus transmission was precluded. Spindler *et al*. (1946) showed that the feces and/or urine from animals fed infected swine tissue was capable of producing sarcocysts in previously uninfected swine. Unfortunately their observation was not pursued.

Fayer (1970, 1972) reported that *Sarcocystis* cystozoites underwent development in cultured cells routinely used to study intracellular development of classical coccidians (*Eimeria* & *Isospora*). This led Fayer (1972) to suggest that *Sarcocystis* might be found to have a two host life cycle similar to that recently discovered for another cyst-forming coccidian *Toxoplasma gondii* (Hutchinson 1965, Work & Hutchinson 1969). Completion of a *Sarcocystis* life cycle was reported by Rommel and Heydorn (1972) who found coccidian sporocysts in the feces of cats fed sarcocyst infected sheep muscle. Subsequent efforts (Heydorn & Rommel 1972, Mahrt 1973, Fayer 1974, Rzepczyk 1974, Munday et al. 1975, Zaman & Coley 1975, Dubey 1976) have substantiated the initial results. Mammalian Sarcocystis is now thought to have an obligate two host life cycle with asexual multiplication and cyst formation in a herbivorous intermediate host followed by gamete formation and sporogony in the intestinal tissues of a carnivorous definitive host.

Summarizing their series of excellent studies, Mehlhorn et al. (1975) revealed that ultrastructurally distinguishable cysts from the same intermediate host were capable of infecting different definitive host species. They also found that a single definitive host species could be infected by cystozoites originating in different intermediate host species. These life cycles were found to be consistent; each cyst type from the intermediate host species was infective to only one definitive host species and sporocysts from those infections resulted in the original cyst morphologies developing in animals of the original intermediate host species. Cyst ultrastructure and completion of the life cycle have become fundamental to the taxonomic designation of each species. The previous taxonomic scheme of one host-one species is now considered invalid. Proposals by Heydorn et al. (1975) which have been clarified recently by Levine (1977) reflect the application of this new awareness to nomenclature within the genus.

Fayer and Johnson (1973, 1975) described the development of muscle cysts in calves fed sporocysts recovered from the feces of dogs previously fed Sarcocystis infected beef. Prior to the appearance of the sarcocysts large numbers of coccidian-like schizonts were found in a wide variety of host tissues (Fayer & Johnson 1973, Johnson *et al*. 1975). Johnson *et al*. (1975) further reported that the host immunologic response to the developing schizonts resulted in extensive lesions which were accompanied by serious illness and often death of the intermediate host. Similar results were reported by Hudkins and Kistner (1977) for deer fawns (*Odocoileus hemionus*). Fayer (1975) reported that pregnant cows fed *Sarcocystis* sporocysts became clinically ill and aborted. The foetuses were parasite free; thus negating the possibility of transplacental transmission.

Several big game species in Alberta have been examined for the presence of *Sarcocystis*. Deer (*O. hemionus*, *O. virginianus*), moose (*Alces alces*), elk (*Cervus canadensis*), bighorn sheep (*Ovis canadensis*), and Rocky Mountain goat (*Oreamnos americanus*) have high prevalences of infection (Mahrt & Colwell, unpublished data). Little is known about the life cycle or taxonomy of *Sarcocystis* from the above hosts with the exception of a life cycle completion (deer-coyote-deer) reported by Hudkins-Vivion *et al*. (1976) and Hudkins & Kistner (1977). Cyst morphology and the development of the parasite in the definitive host are unknown for any big game *Sarcocystis*.

The role of *Sarcocystis* as an agent of disease in both domestic and wild ungulates is now recognized (Corner *et al*. 1963, Fayer 1974, Dubey 1976, Hudkins & Kistner 1977). The disease potential prompted this study of some aspects of the biology of moose *Sarcocystis*. The research was pursued with regard to determining the definitive host(s), elucidating the development of the parasite within the tissues of the definitive host, and identification of the species of *Sarcocystis* present in moose through ultrastructural study of the cyst morphology.

Moose Sarcocystis was selected for study because of the presence of macroscopic cysts in the muscles of the esophagus, tongue and diaphragm. Diagnosis of the infection was easily made in the field and fresh samples could be returned to the laboratory for feeding experiments and ultrastructural studies.

CHAPTER II METHODS AND MATERIALS

The muscle samples used during this study, either for histological examination or for feeding experiments, were acquired in various ways. Tongues and occasionally esophagi were obtained from hunters at a check station maintained in the Swan Hills area during the fall of 1975. Entire carcasses of "road killed" moose were placed at our disposal throught the cooperation of the staff of Alberta Parks, Recreation and Wildlife. A permit granted to J. L. Mahrt by Alberta Parks, Recreation and Wildlife allowed the collection of a single adult moose in February, 1976. In addition, tongue, esophagus, diaphragm and heart of several moose were received from members of independent hunting parties.

PREVALENCE AND CYST MORPHOLOGY

Gross examination of muscle samples for the presence of sarcocysts was followed by fixation of a portion of each sample in 10% neutral buffered formalin. Enough material was fixed from each tissue so that at least one cm^2 of tissue could be examined in histological section. Samples for fixation were taken from the same location whenever possible. In the case of the tongue, samples were taken at the midline near the tip and from a ventro-lateral muscle mass near the base. Tissue from the heart was taken at the apex. Tissues from the esophagus, diaphragm and the skeletal musculature were taken at random.

Sections of paraffin-embedded muscle tissue were cut at 6-8 micrometers, stained with Whipf's polychrome (Vetterling & Thompson 1971), or with Heidenhain's Iron Haematoxylin and scanned using 10X or, 16X objectives on a Zeiss Photomicroscope. Further light microscope observations on cyst structure were made using 40X oil and 100X oil immersion objectives.

When it could be ascertained that the death of the moose was recent (i.e., less than 8 hr), cysts were dissected free with a small amount of surrounding muscle and fixed in one of the following fixatives: 3% glutaraldehyde (PO_4 buffer pH 7.4) or 3% acrolein (PO_4 buffer pH 7.4). All samples were then washed in buffer, post-fixed in 1% osmium tetroxide, block-stained in saturated aqueous uranyl acetate (70°C, 15 min.), dehydrated in ethanol and embedded in Spurr's low viscosity epoxy (Spurr 1969). Thick sections (1 micrometer) were stained with Toluidine Blue O (1% aqueous with 1% borax). Thin sections, cut with glass knives on a Sorval MT2 ultramicrotome, were mounted on Formvar coated 200 or naked 400 mesh copper grids. Sections were stained with 1% lead citrate or sequentially with 1% potassium permanganate and 1% lead citrate and were observed with a Phillips EM 201 transmission electron microcope at an accelarating voltage of 60 Kv.

LIFE CYCLE AND DEVELOPMENT

Completion of the Sarcocystis life cycle was attempted with dogs, coyotes and cats of known origin. All of the experimental animals were coccidia-free at the beginning of the experiments.

The experimental dogs were beagle pups obtained from breeding stock maintained in the Biosciences Animal Services (BSAS) facility. All dogs (breeding stock and experimental pups) were reared in a coccidia-free environment. Pups, when weaned, were kept in individual stainless steel cages. Prior to the initiation of experimentation the feces of each dog was examined weekly for the presence of coccidian oocysts or sporocysts.

Three coyotes were obtained by Caesarean section from a hunter killed female. The coyote pups were hand raised by BSAS staff. The feces of the individually caged animals was examined at infrequent intervals until experimentation was begun.

Four cats were brought to the BSAS facility when they were approximately four months of age. Upon arrival they were passing large numbers of Isospora felis oocysts. Treatment with a coccidiostat (Sulfaquinoxaline) was initiated and this presumably terminated oocyst output. Feces from each cat was then checked weekly until the experiments were begun.

Experimental infections were conducted in four distinct, but somewhat overlapping aspects. Each experiment was designed to look at a particular portion of the life cycle and development in the definitive host. Details of each aspect are outlined below and summarized in Table 1.

(I) Prepatent Period and Sporogony

Two cats and four dogs were fed freshly ground moose muscle (heart, diaphragm, skeletal muscle) for three or four consecutive days. One coyote was fed ground tissue from the tongue of a single infected moose. Control animals (one of each species) were maintained in the same room as each of the experimental animals, but were not exposed to infective material. The feces of all animals was examined daily, for 30 days or until the animal was filled according to the following protocol. Feces collected each morning was homogenized in 200 ml of aqueous potassium dichromate (2.5%) and approximately six ml of the resultant slurry was strained through two layers of cheesecloth into a 15 ml centrifuge tube. Saturated sugar solution (sp. gr. 1.27) was added until a slight positive meniscus was achieved. An 18 mm^2 coverslip was gently lowered onto the tube which was then centrifuged at 1100 rpm

Table 1 - Summary of Experimental Infections of Canids and Felids with *Sarcocystis* from moose (*Alces alces*)

Expt. No.	Host	No. fed Infected Tissue	No. of Controls	Tissues Fed	No. of Days Fed	Fecal Exam. (Days)	Necropsy
	Canis latrans	1	1	T*	1	30	-
	Felis domestica	2	1	HDS	3	30	-
I	*Canis familiaris* (1)	4 (1)	1	HDS	3	11	11 days
		(2)			3	12	12 days
		(3)			3	14	14 days
		(4)			4	15	15 days
II	*C. familiaris*	4 (5)	1	HDS	4	4	4 days
		(6)			4	7	7 days
		(7)			4	8	8 days
		(8)			4	9	9 days
III	*C. familiaris*	2 (9)	1	HDS	1**	1	24 hr
		(10)			1**	1	48 hr
IV	*C. familiaris*	3 (11)	1***	EDT	-	-	18 hr 18 hr
		(12)			-	-	18 hr 18 hr
		(13)			-	-	24 hr 72 hr

* T - tongue
HDS - heart, diaphragm, skeletal musclulature
EDT - esophagus, diaphragm, tongue

** Fed to satiation twice in 12 hr period

*** Control tissue taken from uninvolved portions of intestine for ten minutes. After centrifugation the coverslip was raised from the tube and placed on a glass microscope slide. Each coverslip was completely scanned microscopically with a 16X objective. When sporocysts or oocysts were encountered they were measured using an ocular micrometer. Photographs were taken using Kodak Panatomic X film.

Detailed study of the developmental stages of the parasite was achieved by killing the experimental hosts shortly after the onset of sporocyst production or one day prior to the earliest expected date of patency, as determined from the previous animals (see Table 1). The experimental hosts were killed by electrocution. The small intestine was quickly removed, laid out linearly and duplicate segments were removed at 10 cm intervals. Samples were also taken from the cecum, large intestine and mesenteric lymph nodes. The removed intestinal sections were split longitudinally, inverted and fixed, along with the other tissues, in Helly's or Susa's fixatives for light microscopy or in chilled (4°C) 3% glutaraldehyde (PO_4 buffer pH 7.4) for electron microscopy. Mucosal scrapings from each intestinal segment were examined by phase contrast microscopy. Observations were made on the position and relative number of the parasite stages present.

(II) Gametogenis and Early Sporogony

In the second experiment four dogs were fed Sarcocystis infected meat for four consecutive days. On days 4, 7, 8, and 9 post-infection (i.e., the number of days being calculated from the first day of feeding) an experimental dog was killed. Tissues were recovered and treated in the same way as in the first experiment. A control animal was maintained and examined for the presence of coccidians for 30 days at which time it was killed and tissues were taken for histological examination.

(III) Early Gametogenesis

In the third experiment two dogs were fed ground moose meat, until they were satiated, twice within a 12 hr period. The first dog was killed 24 hr post-infection, the second was killed at 28 hr post-infection. Tissues were handled as previously described. These infections were run concurrently with those of experiment II and the control animal from that series served the same role in this portion of the study.

(IV) Surgical Isolation of Intestinal Segments

This work was undertaken in an effort to concentrate high numbers of early gametocytes in a known, relatively small portion of the intestine. This would facilitate the location of the parasites for electron microscopy.

Two segments of duodenum (5-8 cm long) were isolated, with the blood supply intact, from the small intestine of three experimental pups. The length of the isolated segments was dictated by the necessity of maintaining normal blood supply to the tissue. Open ends of the isolates were closed and 1-1.5 ml of a concentrated suspension of cystozoites was injected into the lumen of each. Integrity of the intestine was restored by anastomosis of the free ends.

Cysts dissected with little or no adherant muscle tissue were placed in sterile Earle's saline and disrupted in a teflon tissue grinder. The free cystozoites were pelleted by light centrifugation. The above procedure was used to prepare cystozoites for all of the intestinal isolates except one. In this particular case large pieces of infected muscle were placed in a pepsin-HCl digestion fluid, macerated in a Sorval Omnimixer and warmed to 37°C for 30 minutes. Light centrifugation produced a pellet of cystozoites, essentially free of debris, which was washed several times in Earle's saline to remove the digestion fluid. The morphology of the cystozoites prepared in this way was normal when examined by phase contrast microscopy.

The first two pups which underwent surgery were killed 18 hr post-infection and the intestinal isolates were removed. The third dog was anesthetized at 24 hr post-infection and one isolate was removed. At 72 hr this same dog was killed and the second isolate removed. Each isolate and a portion of the uninvolved intestine from each pup was fixed and processed as described above.

CHAPTER III RESULTS

PREPATENT PERIOD AND DISTRIBUTION OF PARASITES

The coyote and the cats which were fed sarcocyst infected meat passed no oocysts or sporocysts in their feces during the 30 day period post-infection. The feces of the control animals remained negative for oocysts or sporocysts during the same period.

Table 2 summarizes the data on the prepatent period and the distribution of parasites along the length of the small intestine, as determined by examination of fresh mucosal scrapings. Oocysts, in various states of sporulation, were found throughout the length of the small intestine. The heaviest concentrations of parasites were in the anterior half of the intestine and few were found in the 20-40 cm anterior to the ilio-cecal junction. No parasites were observed in mucosal scrapings from the cecum or large intestine of any of the 3 dogs. Figure 1 shows an example of the distribution of oocysts in villi scraped from the intestinal mucosa. Only rarely were oocysts found below the luminal half of a villus.

The sporocysts observed on the first day of the patent period in each dog contained an amorphous granular mass (Fig. 2). No discernible sporozoites were present and the granular mass was more diffuse than the residual body seen in fully sporulated sporocysts (Fig. 3) observed on later days. After the second day of the patent period only fully sporulated sporocysts were observed in the feces. Occasionally two sporocysts were found in close proximity to one another; careful observation revealed the presence of a thin oocyst wall adherant to the sporocysts (Fig. 2).

Table 2 - Prepatent Period and Distribution of Parasites

Experiment	Dog	Prepatent Period (Days)	Parasite Distribution (cm. from pylorus)	
			Total Distribution	Area of Concentration
I	1	11	10-110	50-70
I	2	12	10-140	20-70
I	3	15	10-160	20-70

Sporulated sporocysts found both in situ in the intestinal mucosa and in the feces contained four fusiform sporozoites and a compact sprorocyst residuum (Fig. 1 & 3). The residuum had a distinct boundary and appeared to be membrane bound. The sporocysts measured 11.4 um x 15.6 um (10.8-11.5 x 14.5-15.8, n=20).

ENDOGENOUS STAGES I: GAMETOGONY

Histological examination of the intestinal isolates and of tissues from the pups fed sarcocyst infected meat revealed the presence of microgametocytes, macrogametes and oocysts in various stages of formation and sporulation (Table 3).

Parasites occupied cells of the intestinal lamina propria which were immediately below the epithelial layer. Each parasite occupied a distinct parasitophorous vacuole. Distortion of the host cells caused by the parasitophorous vacuole made identification of the parasitized cell type impossible. With the exception of the parasites found in tissue from the intestinal isolates (Expt. III), which occupied cells in the tunica propria surrounding the crypts of Leiberkuhn, all of the parasites occupied cells in the liminal half of the villi.

The distribution of parasites along the length of the small intestine was the same as that observed during examination of the fresh preparations. No parasites were found in sections of tissue from the cecum, the large intestine or the mesenteric lymph nodes. Tissue from the control pups and uninvolved segments of intestine from the pups with intestinal isolates were found to be completely parasite free.

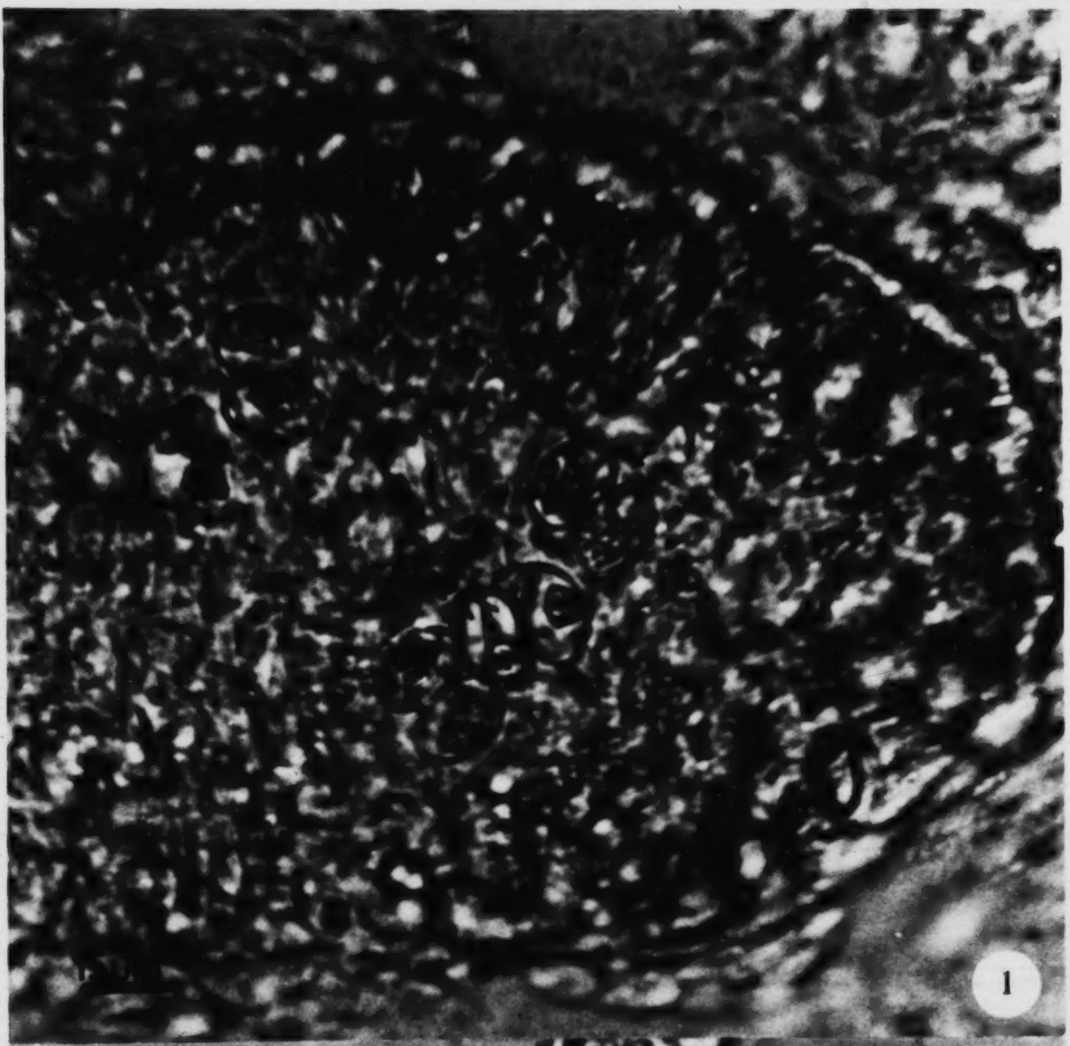
Microgametocytes and early macrogametes were extremely difficult to find and only a very few of them were located in either orally infected pups or in the intestinal isolates. Microgametocytes were

Figures 1 - 3. Photomicrographs of sporocysts from fresh mucosal scrapings or from feces of dogs fed sarcocyst infected moose meat.

Figure 1. Tip of villus from fresh mucosal scraping. Note paired sporocysts with evidence of oocyst wall (arrows). (sr = sporocyst residuum, sp - sporozoite). Phase contrast microscopy.

Figure 2. Pair of unsporulated sporocysts found on the first day of the patent period. Note large granular mass in sporocysts, the absence of sporozoites and the presence of a remnant of the oocyst wall (arrow).

Figure 3. Fully sporulated sporocyst from feces of an experimentally infected dog. (sr = sporocyst residuum, sp = sporozoite).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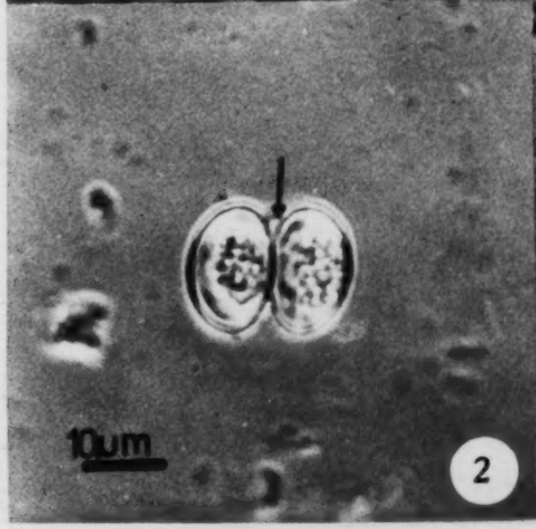

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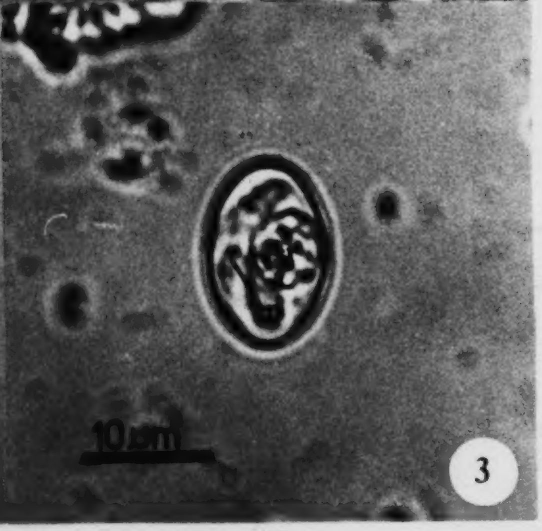

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Table 3 - Summary of Endogenous Stages Seen in Histological Sections of the Intestine of Experimentally Infected Dogs

Experiment	Dog No.	Time of Necropsy	Developmental Stages								
			Micro-Gametocyte	Micro-gamete	Macro-gamete	Oocyst	Sporogonic Stages				
							1st Division	Binucl. Sporont	Uninucl. Sporoblast	Binucl. Sporoblast	Sporulated
IV	13	18 hr	+		+						
IV	*	18 hr									
IV	14	18 hr	+		+						
IV		18 hr	+		+						
IV	15	24 hr									
IV		72 hr									
II	6	24 hr	+		+						
II	7	48 hr	+		+						
III	8	4 days			+	+					
III	9	7 days				+	+				
III	10	8 days				+	+	+			
III	11	9 days				+	+	+	+	+	
III	12	control									
I	1	12 days							+	+	+
I	2	14 days								+	+
I	3	15 days							+	+	+
I	4	11 days				+	+	+	+	+	+
I	5	control									

identified by the presence of small dark staining bodies thought to be the nuclei of developing microgametes. Various stages of microgametocyte development were observed (Fig. 4 & 5) but no fully formed microgametes were observed.

Accurate identification of the early macrogametes was difficult. A large number of the parasites observed in tissues from pups in experiments II and IV had a single dark staining body (nucleus) and several large oval granules in the cytoplasm (Fig. 6); both features characteristic of macrogametes.

ENDOGENOUS STAGES II: SPOROGONY

The first fully formed unsporulated oocysts were observed 7 days post-infection (p.i.) (Fig. 7) (see Table 3). From that time on oocysts in various stages of sporulation were easily observed. Detailed observations on tissues from Experiments I and III allowed construction of the following sequence of events. The unsporulated oocyst, with its thin oocyst wall, contains a single large, uninucleate zygote; the sporont (Fig. 8). The first observable event in the process of sporogony is lengthening of the sporont nucleus to produce a "nuclear streak" (Fig. 9). Oocysts with a prominent "nuclear streak" were first found in the tissue 7 days p.i., but because of the multiple feedings of each pup these stages were also present at 8, 9 and 11 days p.i.. Eight days p.i. oocysts with binucleate sporonts were first observed and occasionally oocysts were found in which the binucleated sporont showed beginnings of cytokinesis (Fig. 10). At 9 days p.i., in addition to the earlier described stages, oocysts were observed in which the cytokinesis was complete; present in these oocysts were two uninucleate cells (sporoblasts) (Fig. 11). On days 11 and 12 numerous oocysts

Figures 4 - 13. Endogenous stages of Sarcocystis in the intestinal lamina propria of dogs fed moose muscle.

Figure 4. Microgametocyte in tissue of intestinal isolate 18 hours after inoculation. Heidenhain's Iron Haematoxylin. (HIH)

Figure 5. Elongate microgametocyte in lamina propria of a dog fed infected muscle. 48 hours p.i. HIH

Figure 6. Macrogamete 4 days p.i. HIH.

Figure 7. Macrogamete 6 days p.i. HIH.

Figure 8. Oocyst 7 days p.i. HIH.

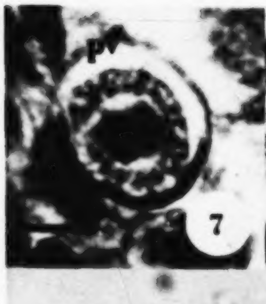
Figure 9. Oocyst; sporont undergoing first nuclear division. Note elongate nucleus ("nuclear streak"). HIH.

Figure 10. Oocyst; sporont undergoing a cytokenesis. Whipf's polychrome.

Figure 11. Oocyst; 2 uninucleate sporoblasts. HIH.

Figure 12. Oocyst; second nuclear division complete. HIH.

Figure 13. Fully sporolated oocyst. Whipf's polychrome.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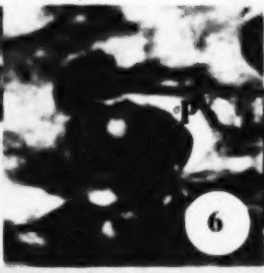
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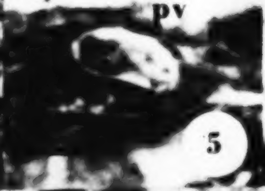
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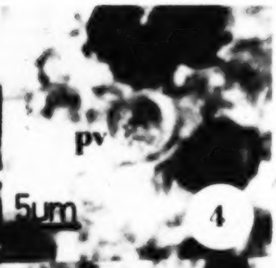
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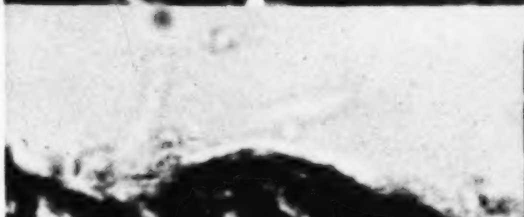
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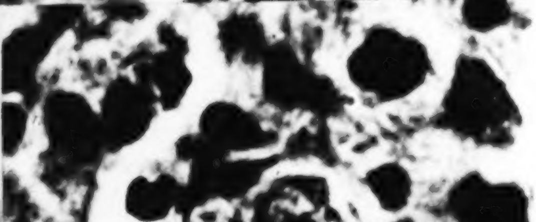
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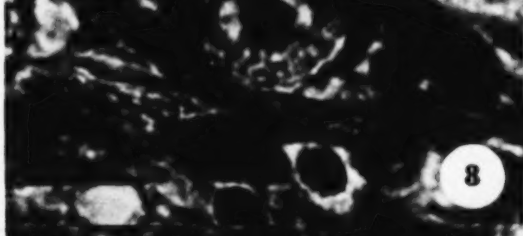
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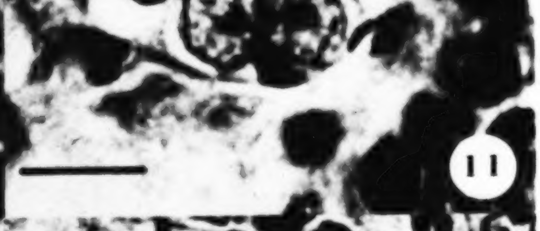
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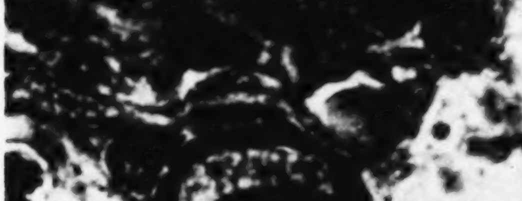
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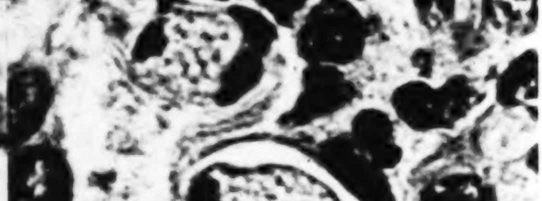
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were observed which contained two binucleate sporoblasts (Fig. 12). Beginning with day 12 p.i. and increasing in numbers through days 14 and 15 there were fully sporulated oocysts present in the intestinal tissues (Fig. 13). Wrinkling of the oocyst wall and sporocysts wall often obscured details of the contents, but it was possible to discern four distinct sporozoites in each sporocyst.

ENDOGENOUS STAGES III: ULTRASTRUCTURE

Electron microscope observations on the parasite stages in the definitive host intestine were limited. Some data was obtained on oocyst wall formation, forming and complete sporocyst walls and on the host tissue surrounding the parasitized cells. No microgametocytes or microgametes were observed.

Parasitized cells were found in the villar lamina propria immediately below the epithelium. Most of the infected cells were in close proximity to capillaries and occasionally the parasite within its distended host cell protruded into the capillary lumen (Fig. 14). Despite the close association with capillaries it does not appear that the parasites were within endothelial cells.

Oocysts in the process of wall formation were observed. Wall forming bodies (WFB) 0.31-0.55 nanometers (nm) in diameter were distributed throughout the cytoplasm. These moderately dense bodies were of generally uniform consistency and were always surrounded by a distinct electron lucid halo (Fig. 15). On several occasions a unique WFB, of distinct shape and size (1.0 um) was noted (Fig. 16). It was associated with a disc shaped structure in the forming oocyst wall (Fig. 16). The forming oocyst wall was variable in structure and width (0.07-0.43 um). In some regions the moderately dense wall was of uniform structure, in others it was reticulated or liminated in structure. Amylopectin and lipid granules were the most prominent features within the sporont although occasionally a nucleus and mitochondria were evident. In sporulated oocysts the completed oocyst wall was thin (89-130 nm) (Fig. 21). In many instances the oocyst wall was not present. The sporocysts were often surrounded by an electron dense, granular material (Fig. 20).

In the parasitophorous vacuole of a few forming oocysts there were a number of long tubular inclusions 67 nm in diameter (Fig. 17). These inclusions were variously oriented but it was noted that their origins were in the host membrane of the parasitophorous vacuole. The developing oocyst in Figure 17 also contains a cross-section of a typical micropore (0.15 um in diameter) common in mature macrogametes.

The forming sporocysts wall (Fig. 18) bounded externally by a single membrane, was variable in thichness (49-152 nm). Internal to the limited membrane is a thick granular layer with irregularly spaced electron lucid areas and large electron dark bodies. The granular zone is separated from the sporoblast cytoplasm by an electron lucid layer and a thin electron dense layer. The separation of the granular zone and the sporoblast cytoplasm in occasionally indistinct. The indistinct separation is usually evident where large dark bodies, surrounded by an electron lucid halo, are close to the surface of the sporoblast. The dark bodies bear a strong resemblance to the WFB of oocysts.

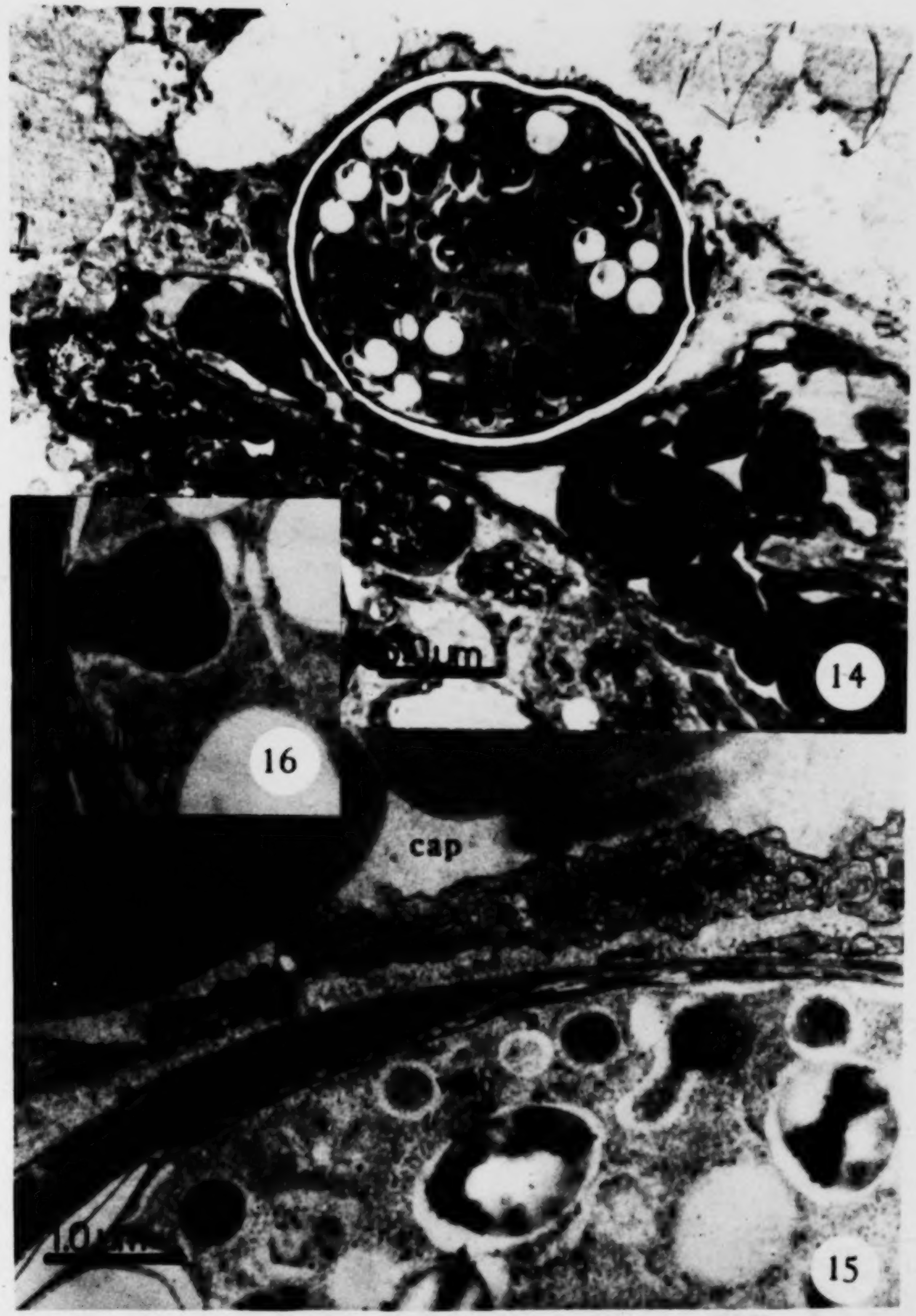
The complete sporocyst wall (Fig. 19 & 20) was composed of an outer uniformly granular layer (56 nm) separated from an inner, denser, non-granular layer (14.5-19.5 nm) by an electron lucid zone (12-18 nm). Beneath the inner layer are two membranes surrounding the sporoblast

Figures 14 - 16. Electron micrographs of endogenous stages of *Sarcocystis* in the intestinal lamina propria of dogs fed moose muscle. (Glutaraldehyde - PO_4)

Figure 14. Oocyst and host cell protruding into lumen of capillary (rbc = erythrocyte).

Figure 15. Developing oocyst wall (arrows) and wall forming bodies (wfb) in sporont cytoplasm.

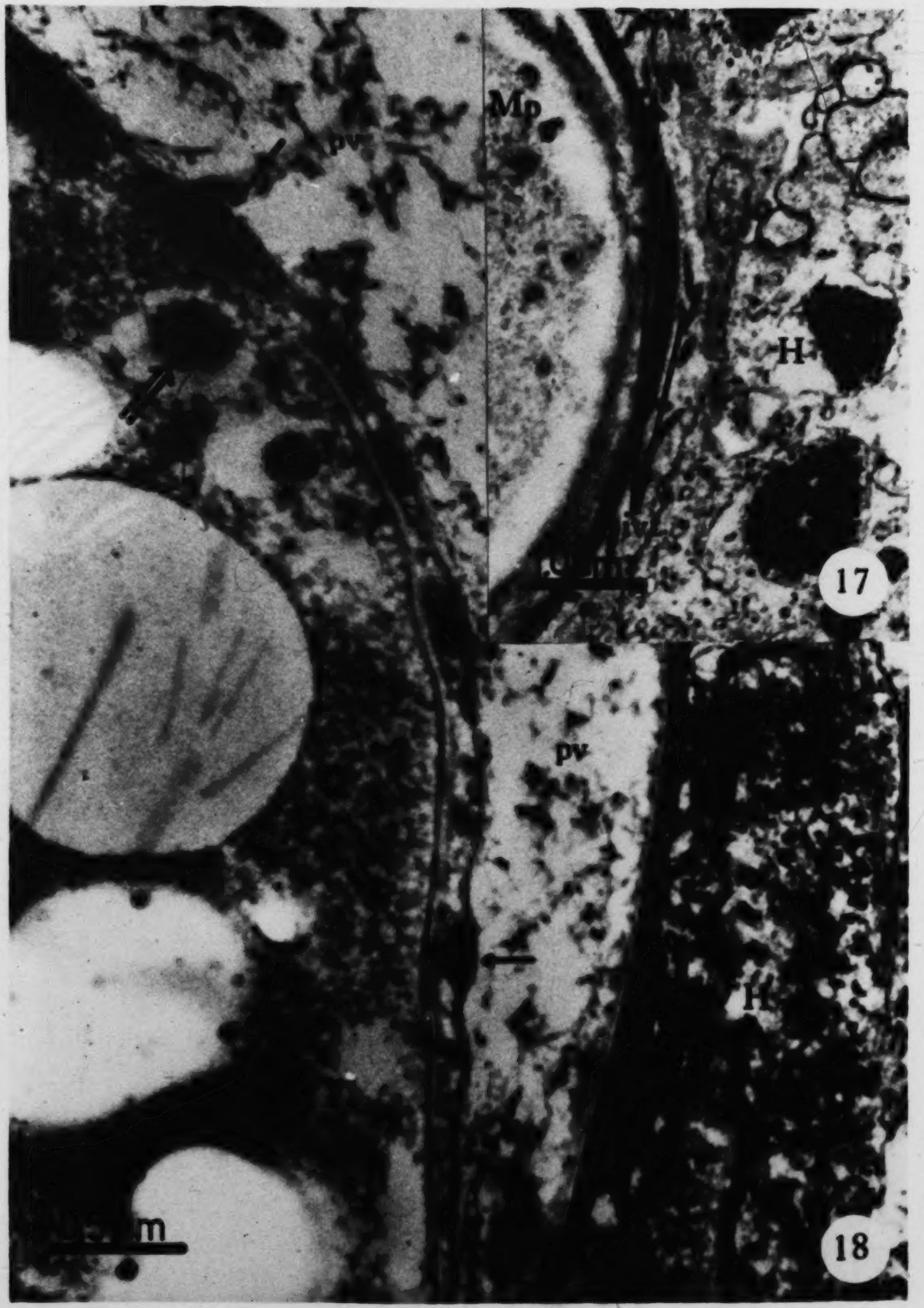
Figure 16. Unique wall forming body (wfb) and associated disc shaped structure (dsc) found in developing oocysts.


14
16
cap
1.0 µm
15

Figures 17 - 18. Electron micrographs of endogenous stages of *Sarcocystis* in the intestinal lamina propria of dogs fed moose muscle (Glutaraldehyde - PO_4).

Figure 17. Developing oocyst in parasitophorous vacuole occupied by numerous intravacuolar tubules (ivt). Note the typical micropore (Mp) present on the sporont surface. (H = host tissue).

Figure 18. Developing sporocyst wall. Note what appear similar to wall forming bodies in the sporoblast cytoplasm (double arrows). The sporocyst wall material has not yet condensed and concentrations of dark staining material are evident (arrows). Note the lack of an oocyst wall and the reticulated contents of the parasitophorous vacuole (pv).



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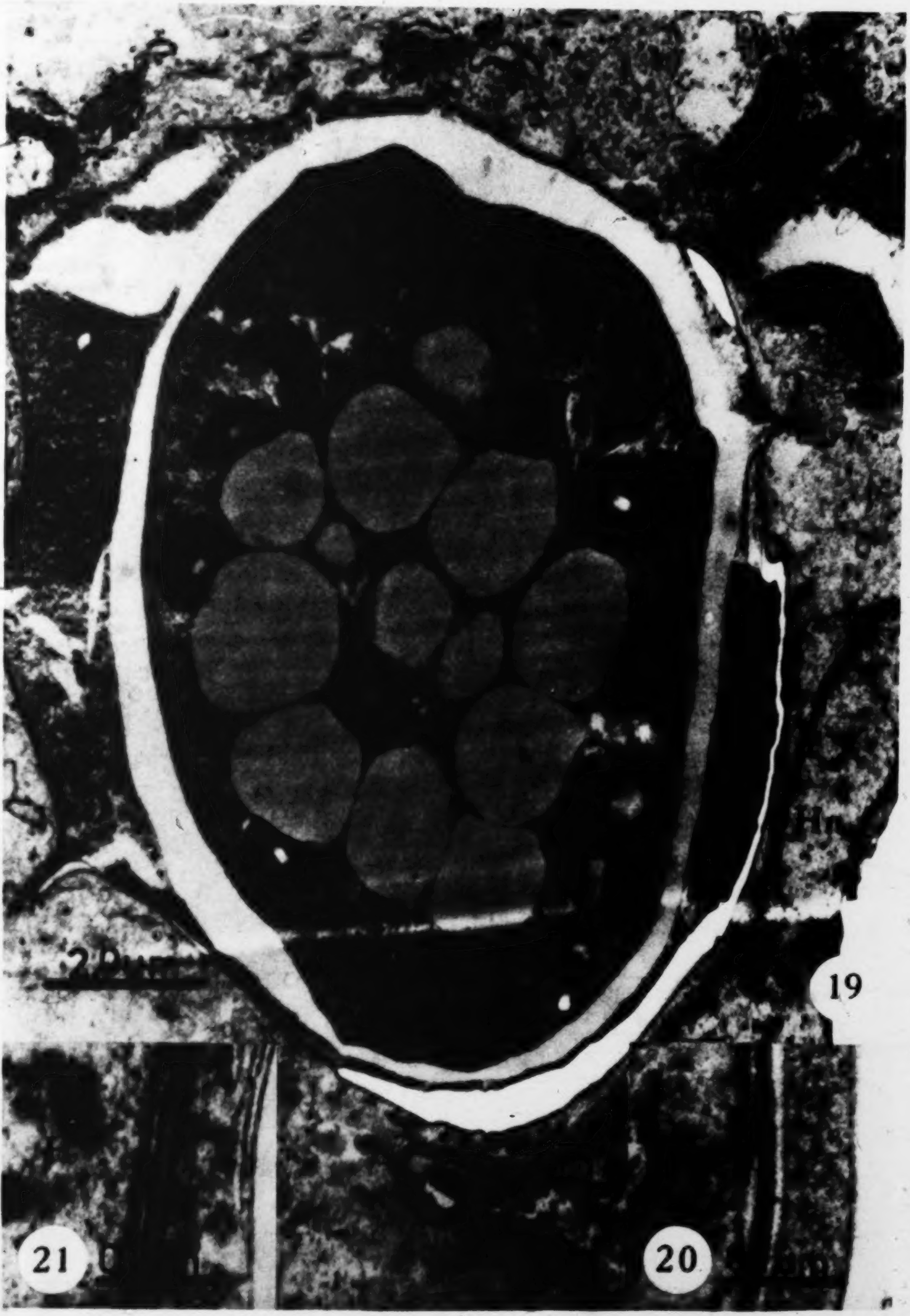
Figures 19 - 21. Electron micrographs of endogenous stages Sarcocystis in the intestinal lamina propria of dogs fed moose muscle. (Glutaraldehyde - PO_4).

Figure 19. Sporoblast at binucleate stage The sporocyst wall is complete. Note the irregular structure of the oocyst wall.

Figure 20. Detail of sporocyst wall: thick outer layer(1), thinner layer(2) and underlying membranes (3).

Figure 21. Detail of oocyst wall: 5 layers present.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cytoplasm. The inner of these two membranes was incomplete. Nuclei, mitochondria, lipid and anylopectin granules were present in the sporoblast cytoplasm.

CYST STRUCTURE: GROSS AND HISTOLOGICAL

Striated and cardiac muscle tissues from 40 adult moose were examined grossly and histologically for sarcocysts. Thirty-two moose (80%) were infected (see Appendix I for detailed data and a comparison of gross and histologic examination results). In 19 of the moose which were diagnosed as positive for Sarcocystis by gross examination there was only one type of cyst noted. These cysts, termed Type I, were fusiform, 1-7 mm long and 0.5-0.75 mm in diameter. A freshly dissected cyst shows a characteristically sculptured surface (Fig. 22). In two moose a second type of cyst (Type II) was present in addition to the Type I. The Type II cysts were spherical to slightly oblong in shape and were 0.5-2.0 mm in diameter. Individual representatives of each type were often in close proximity to one another and were present together in samples of tongue, esophagus and diaphragm. None of the moose examined had only Type II cysts present.

Cyst histo-morphology, as seen by light microscopy, was variable and clear distinctions between Type I and Type II cysts were possible only in those cases where the Type I cysts were sectioned longitudinally. None of the other features noted were consistent enough to allow a safe distinction. All of the cysts (Fig. 23-28) examined had a thin, smooth cyst wall. In some cysts the interior was divided by distinct septa which compartmentalized the tightly packed cystozoites (Fig. 23, 28). In the centre of some of the larger cysts (Fig. 23) there were no cystozoites present although the septa were still visible. Figure 28

Figures 22 - 28. Sarcocysts in a variety of striated muscles from moose.

Figure 22. Surface view of sarcocyst dissected from surrounding muscle tissue. Fresh preparation: tongue.

Figure 23. Longitudinal section through large (macroscopic) cyst from tongue. Note internal septa and central zone devoid of cystozoites. Heidenhain's Iron Haematoxylin (HIH).

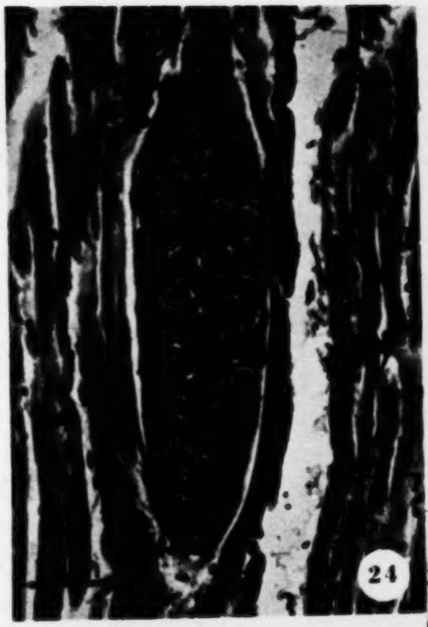
Figure 24. Longitudinal section through cyst in heart. Septa are indistinct. (HIH).

Figure 25. Transverse section of cyst in tongue. No septa apparent. (HIH).

Figure 26. Transverse section cyst in tongue. No discernible septa. (HIH).

Figure 27. Transverse section of small cyst in tongue. Septa not apparent but cyst interior is compartmentalized. (HIH).

Figure 28. Section of very large cyst (macroscopic) from diaphragm. Note clearly defined septa. (This cyst was identified as Type II during gross examination of muscle sample prior to fixation.) (HIH).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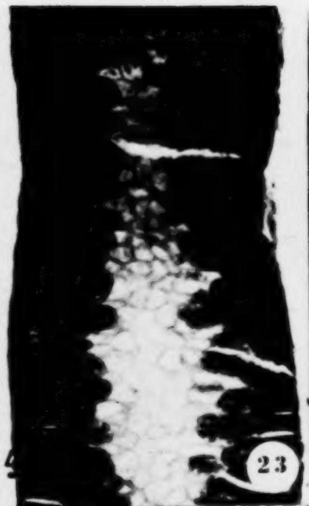
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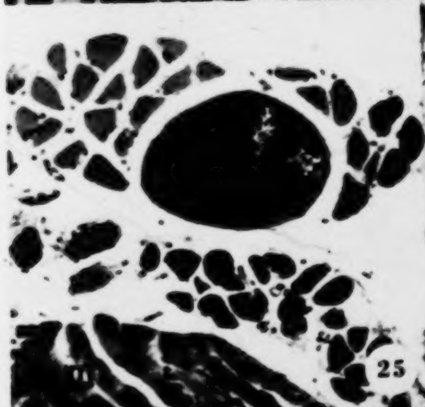
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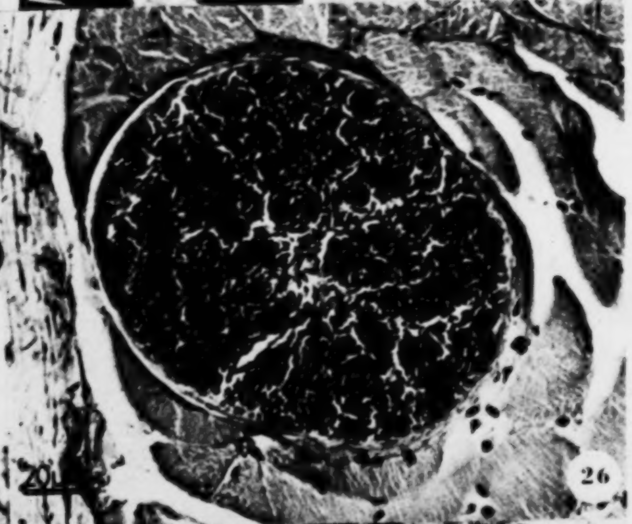

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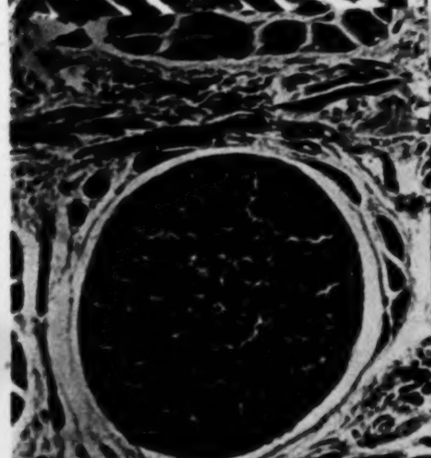
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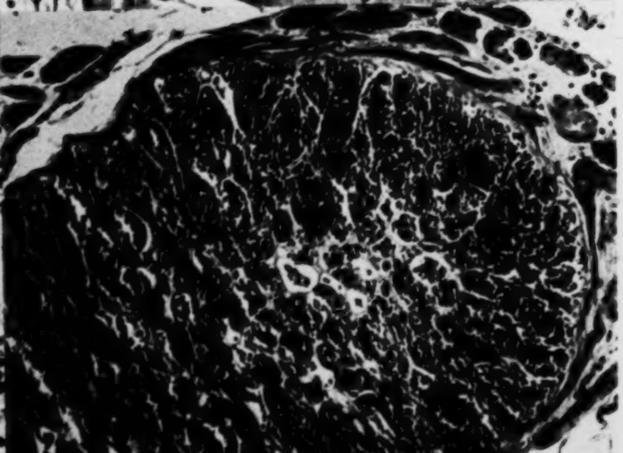
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shows a cross-section of a large cyst with cystozoites present throughout the interior. The large diameter of this cyst suggested that it was a Type II cyst. Figures 24, 26, and 27 show cysts from various tissues whose cystozoites appear to be compartmentalized, but no distinct septa can be seen. A non-compartmentalized cyst interior is seen in Fig. 25. In general the cysts found in cardiac and skeletal musculature had a smaller maximum diameter than those observed in esophageal, lingual, or diaphragmatic musculature. In no case was there any evidence of a host cellular response to the presence of the cysts.

CYST ULTRASTRUCTURE

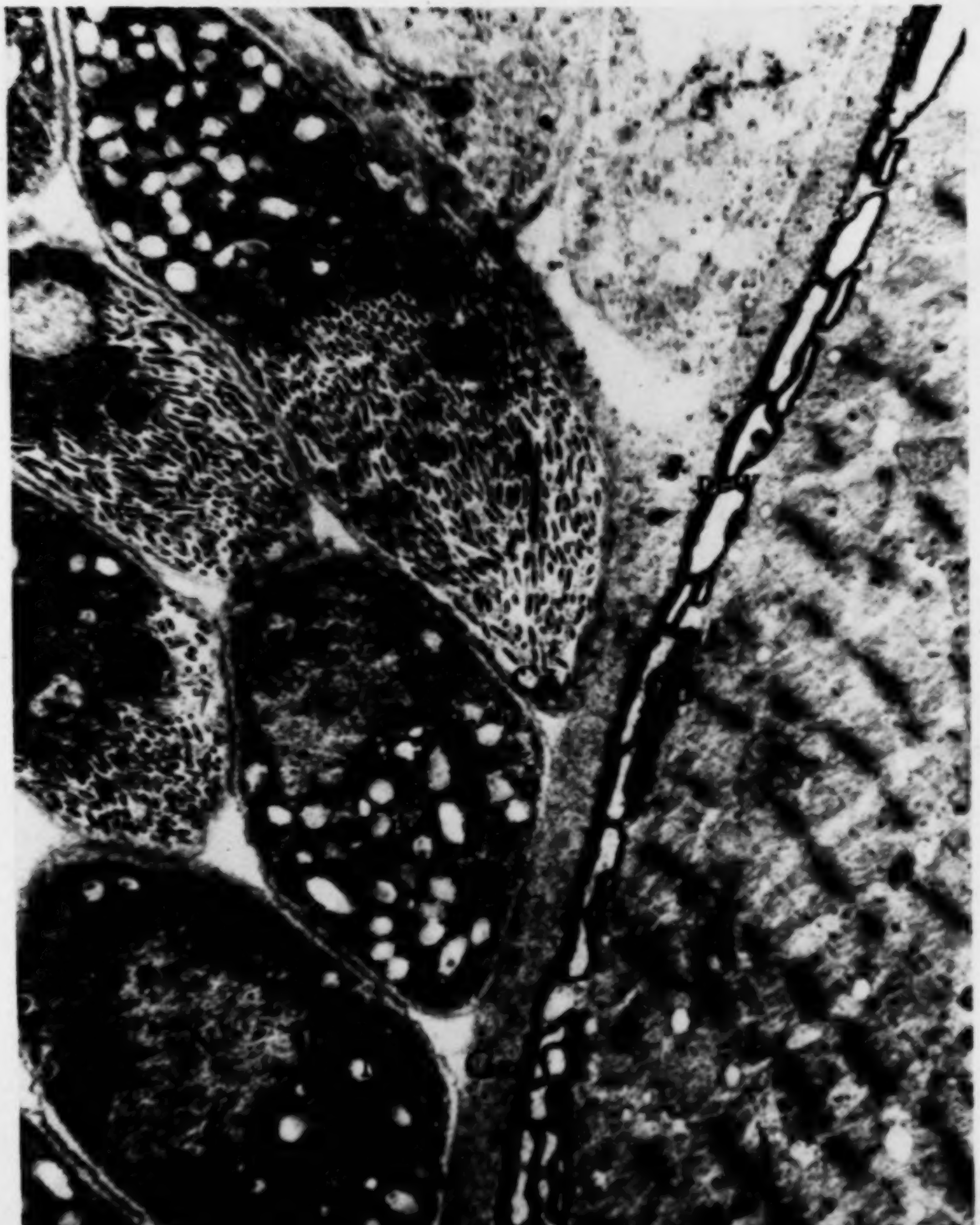
Electron microscope observations were made on the two previously described types of macroscopic cysts and their contents. Cysts from lingual, diaphragmatic and esophageal musculature were studied.

The contents of the Sarcocystis cyst are delimited by two structurally and ontogenetically distinct walls. Based on developmental studies of the cyst wall, Scholtyseck (1963) has proposed a terminology for the structures and their organization. This terminology will be employed, with slight modification, throughout.

Type I:

The general organization of the Type I cyst wall is shown in Figure 29. The inner or primary cyst wall (pcw) was thin (15 nm), electron dense and was folded into regularly occurring digitiform projections 73-126 nm high (Fig. 31). Distinct invaginations and membrane bound vesicles were located at the base of each projection. Also present were thick (25 nm) membranous extensions of the pcw which are highly folded over the entire surface of the cyst giving a multilaminar appearance to the surface. Variation in the pattern of folding

Figure 29. Electron micrograph of Sarcocystis from moose. Low magnification view of Type I cyst wall showing the secondary cyst wall (scw), the primary cyst wall (pcw) with membranous extension, the underlying granular zone (Gz), internal septa (se) and several cystozoites (Rh = rhoptries, Mn = micronemes, Co = conoid, M = mitochondria, N= nucleus). (Acrolein - PO_4)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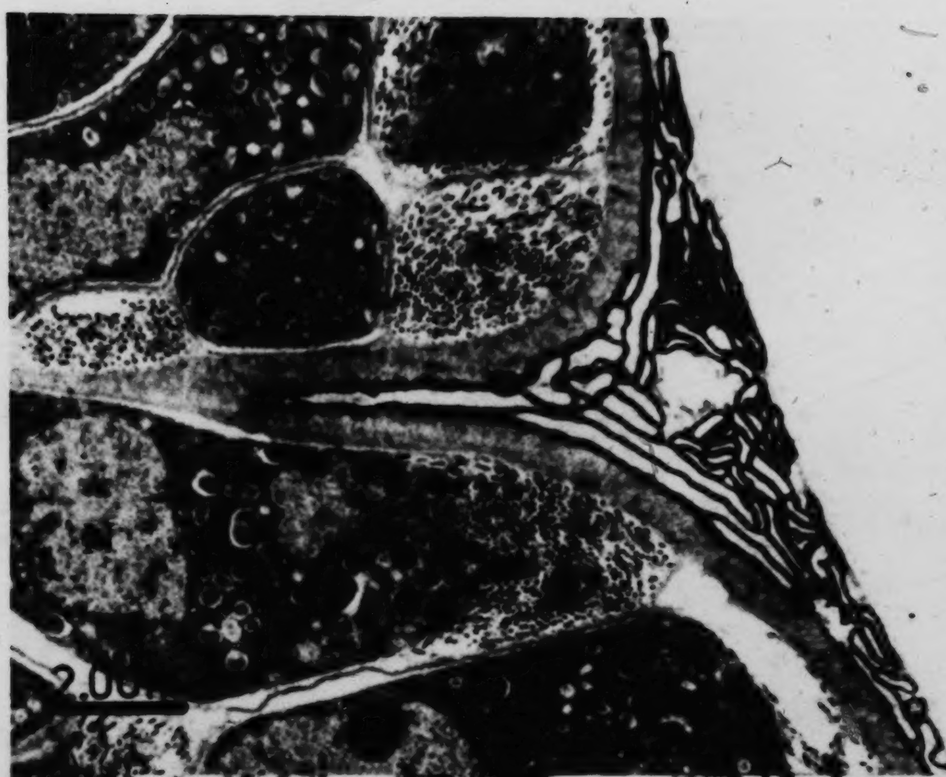
Figures 30 - 31. Electron micrographs of Sarcocystis from moose, Type I cysts.

Figure 30. Low magnification view of cyst wall showing an invagination of the pcw and the highly elaborated membranous extensions of the pcw. (A = amylopectin, N = nucleus). (Acrolein - PO_4)

Figure 31. Cyst wall and cystozoites. Note the flattened membranous extensions of the pcw (double arrow indicates point of origin of a membranous layer), the digitiform projections on the surface of the pcw (arrows). (Acrolein - PO_4)



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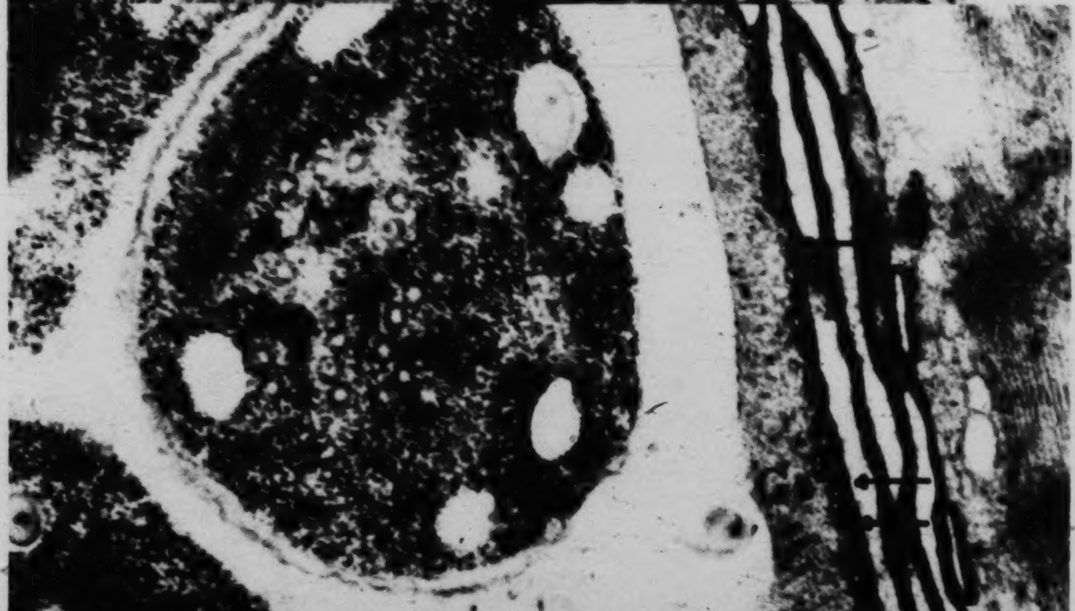

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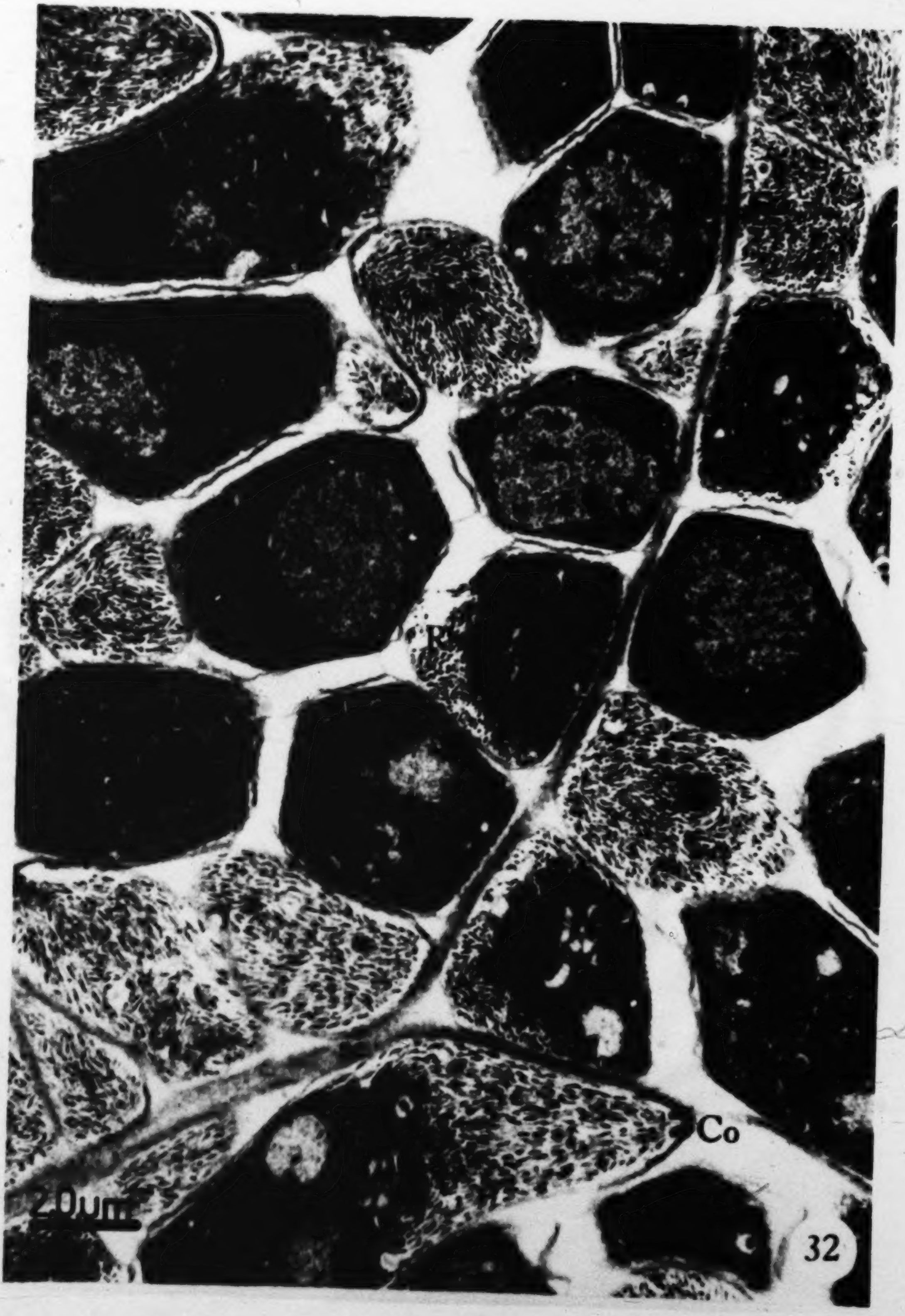
Figure 32. Electron micrograph of Sarcocystis from moose. Low magnification view of the interior of Type I cyst. (Co = conoid, Rh = rhoptries, Mn = micronemes, se = septa, N = nucleus). (Acrolein - PO_4)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Co
20um
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exhibited by these extensions is seen in Figures 30 & 31. Internal to the pcw was a granular zone 420-550 nm thick (Figs. 29, 30 & 31). The granular material did not extend into the interior of the spaces created by the thick membranous pcw extensions. Lying exterior to the thick folded extensions of the pcw was a poorly developed secondary cyst wall (scw). The scw was considered poorly developed because of the lack of fibrillar structures and because of the essentially undisturbed appearance of the nearby host tissue (Fig. 29 & 31). Host mitochondria and nuclei were occasionally found pressed against the outer edges of the thick pcw extensions and rarely a remnant of a host mitochondrion was seen among the thick extensions in close proximity to the actual pcw. At irregular intervals deep invaginations of the pcw and the granular zone produce the previously noted (see Fig. 30) sculpturing of the cyst surface (Fig. 22). The granular zone was confluent with the compartmentalizing internal septa (Fig. 30) at the points where these invaginations occur.

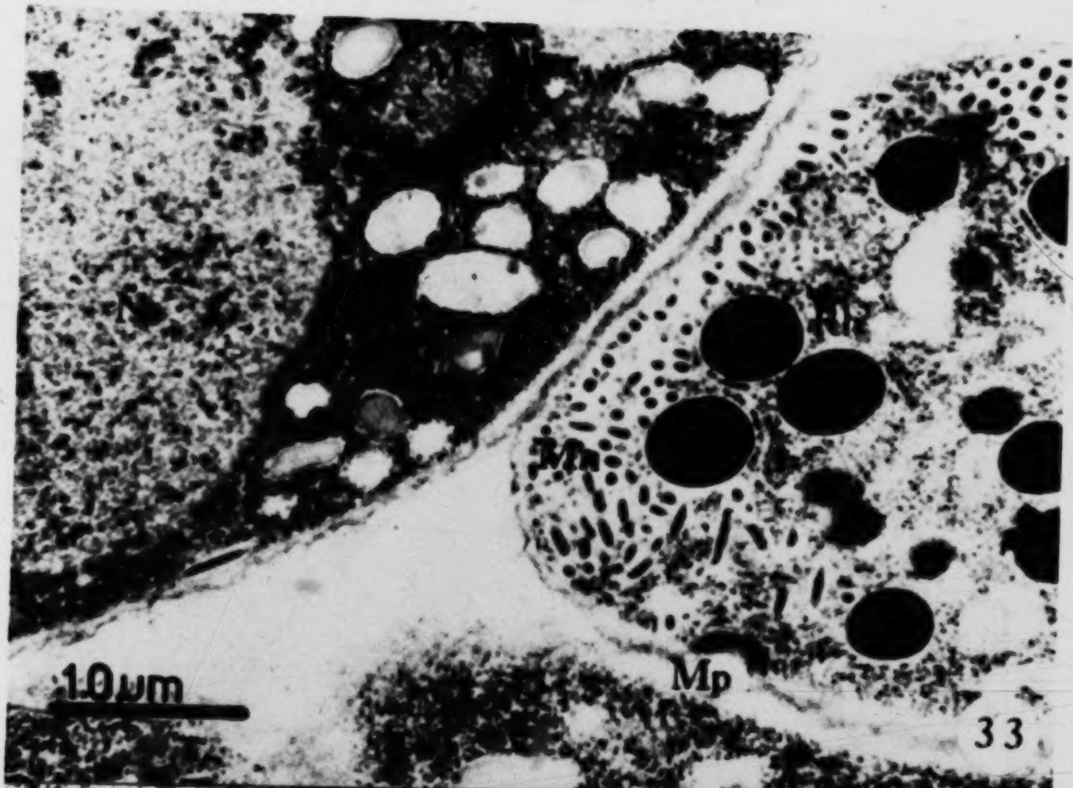
The cystozoites were found tightly packed between the compartmentalizing septa (Fig. 32). It was noted in the Type I cysts that few of the cells within the cyst had a morphology which would have indicated that they were ~~metrocytes~~ (metrocytes are actively dividing precursors of the cystozoites). The outer pellicular membrane of the cystozoites was found to be loosely applied to the inner membrane and, as a result, the outer membranes of adjacent zoites were in close contact (Fig. 33). Cystozoites contained large numbers of micronemes (53 nm dia.) and as many as thirteen rhoptries in the anterior third of the cell (Fig. 32). The posterior two thirds of each organism contained a prominent nucleus, mitochondria, amylopectin granules, rough endo-

Figures 33 - 35. Electron micrographs of Sarcocystis from moose. Details of cystozoites from Type I cysts.

Figure 33. Portions of two cystozoites with detail of mitochondria (M) and closely associated rough endoplasmic reticulum (rer). Also present are rhoptries (Rh), micronemes (Mn), and a micropore (Mp). (Acrolein - PO_4)

Figure 34. Detail of conoid and anterior end of cystozoite. Note polar ring (double arrow) and a subpellicular microtubule attached to it (arrow). (Mn - microneme) (Acrolein - PO_4)

Figure 35. Transverse-section through conoid of cystozoite. Note the polar ring (Pr) and the 22 subpellicular microtubules. Rhoptry necks are seen within the polar ring. (A = amylopectin). (Acrolein - PO_4)


N
Rh
Mn
Mp
1.0µm

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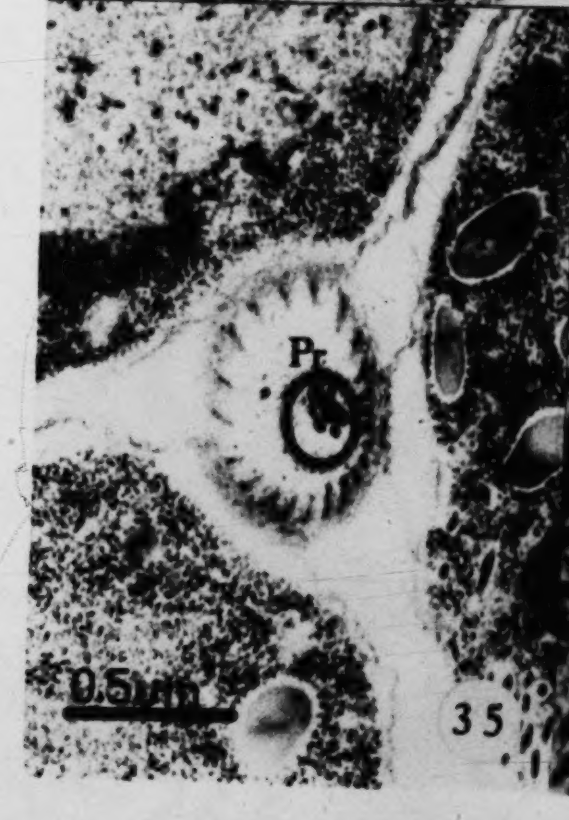

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Figure 36. Electron micrograph of Sarcocystis from moose. Low magnification view of Type II cyst wall showing the inner layer of the secondary cyst wall (scw), the primary cyst wall (pcw), the granular zone (Gz) with connecting septa (se). Also present are cystozoites (cy) and metrocytes (me). (Acrolein - PO_4)

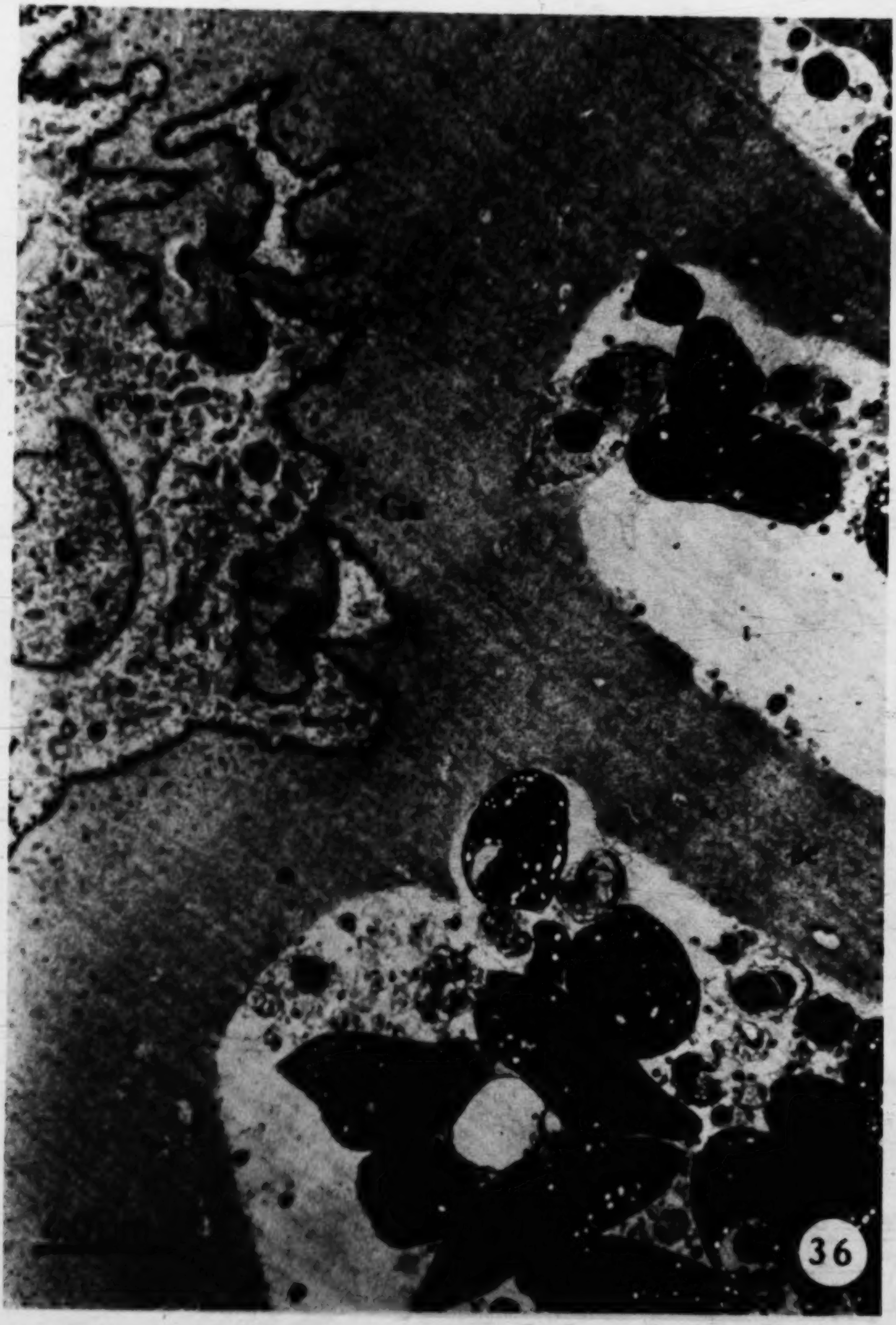

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Figure 37. Electron micrograph of Sarcocystis from moose. Low magnification view of the interior of a Type II cyst. Note variation in septal (se) width. Note also the metrocytes (me); in the upper half a metrocyte is seen with two developing cystozoites, in the lower half an undifferentiated metrocyte is seen. (N = nucleus) (Acrolein - PO_4)

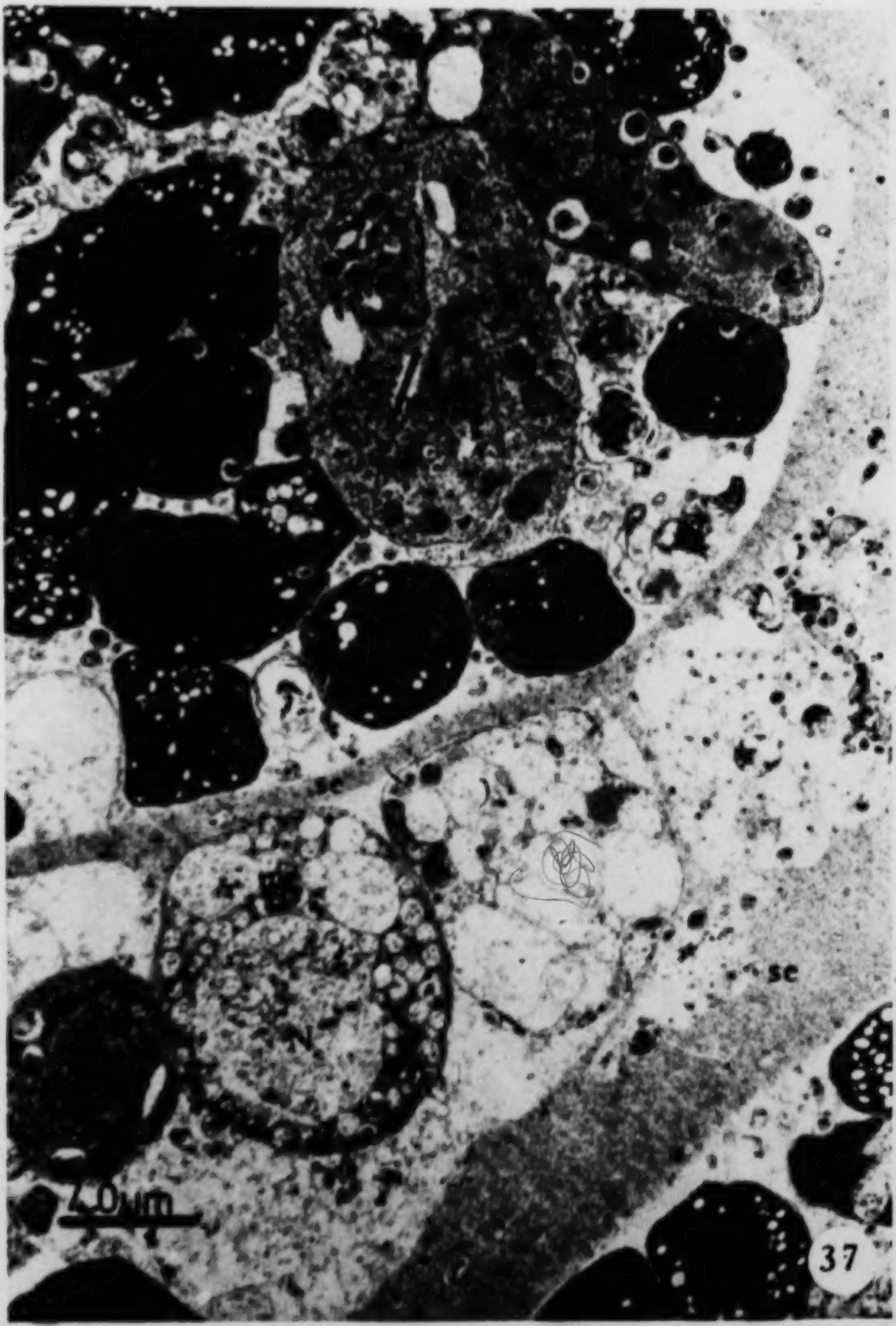
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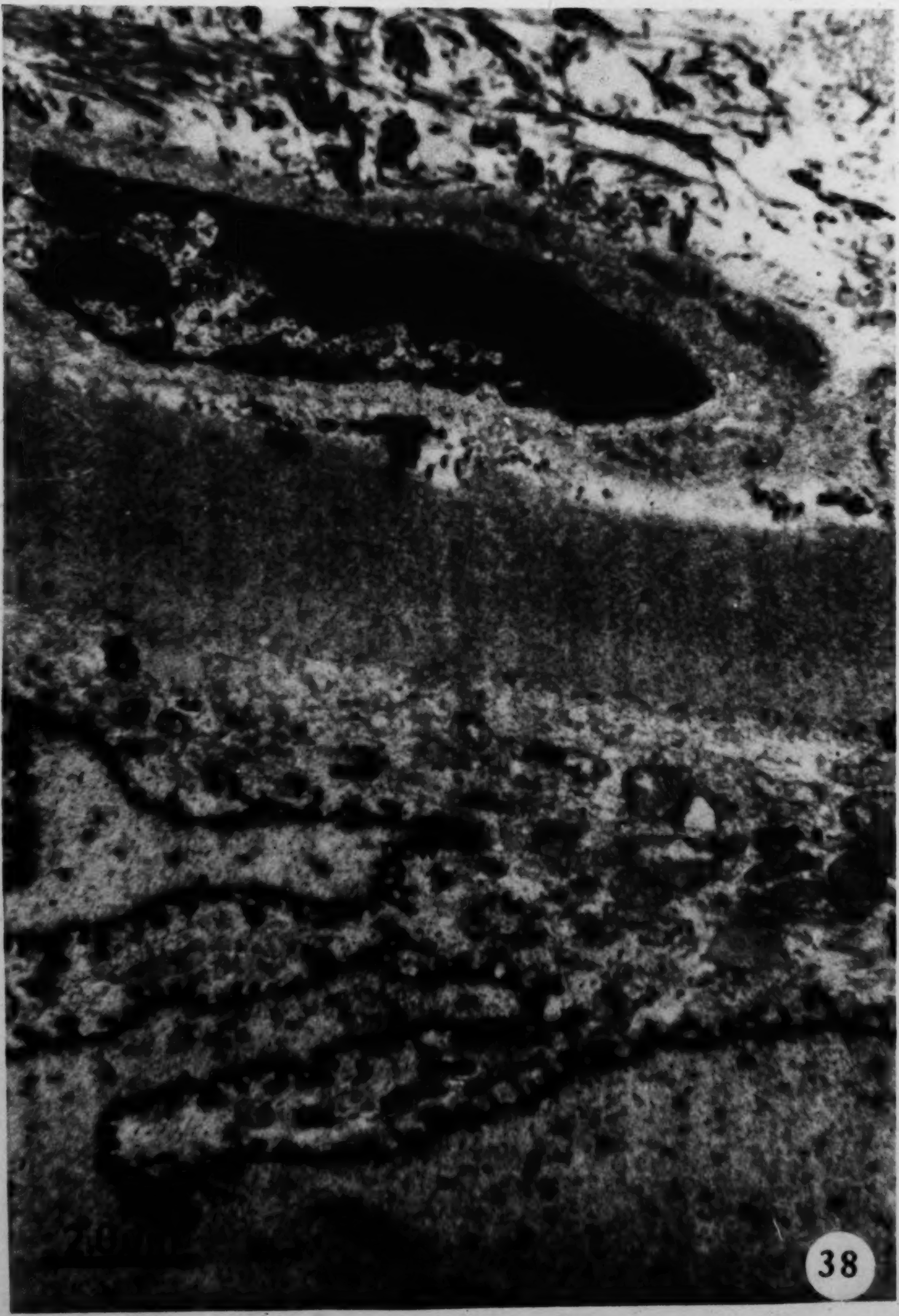
Figure 38. Electron micrograph of Sarcocystis from moose. Detail of Type II secondary cyst wall; three distinct zones are evident. (pcw = primary cyst wall, Hn = host nucleus) (Acrolein - PO_4)



38

plasmic reticulum (rer) and free ribosomes (Fig. 32). The distribution of organelles along the length of each cystozoite probably accounts for the apparent density differences seen in Figure 32. Ribosomes were often noted on the outer nuclear membrane and rer was frequently seen in close association with mitochondria and amylopectin granules (Fig. 33). Micropores were frequently observed in the anterior third of cystozoites (Fig. 33). Typical coccidian conoids, capped with two conoidal rings, were found at the anterior tip of each cystozoite and the necks of two or more rhopties were observed passing through the central lumen of the conoids (Fig. 34). Twenty-two subpellicular microtubules were attached at the polar ring (Fig. 35).

Type II:

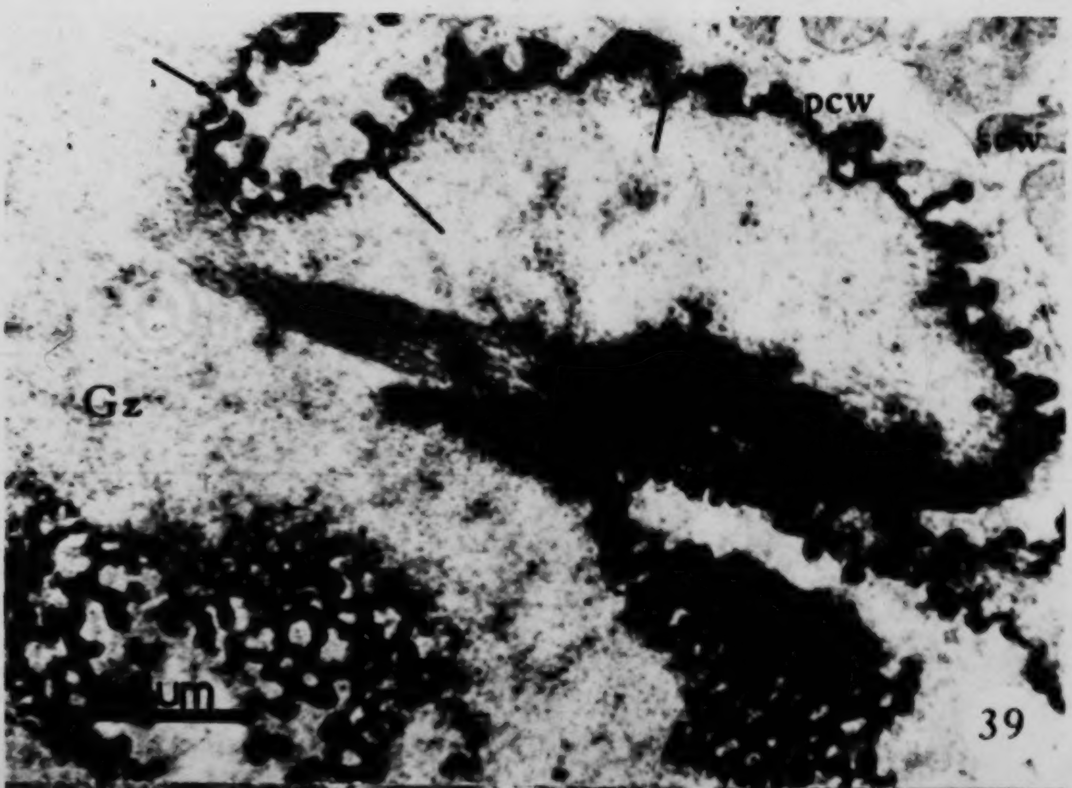
Type II cyst wall organization is shown in Figure 36. The pcw was thin (12 nm) and folded into regularly occuring digitiform projections (185-250 nm in height). The lumen of these projections was filled with a uniform electron dense material. Frequent thinnings or gaps were present in the pcw; these commonly occurred at the bases of the digitiform projections. Superficial sections of the pcw showed that lateral connections between the digitiform projections resulted in a honeycomb-like surface on the cyst. Overall, the pcw was highly folded and branched. Beneath the pcw was a thick granular zone (34-131 um) containing occasional bundles of distinct, regularly oriented fibrils (20 nm in dia.) (Fig. 39). The fibril bundles appeared to have connections with the pcw in at least some of the cases. Granular septa extended from the exterior granular zone into the cyst lumen where they branched to compartmentalize the cyst (Fig. 36). The compartments were loosely packed with zoites, metrocytes and debris (Fig. 37).

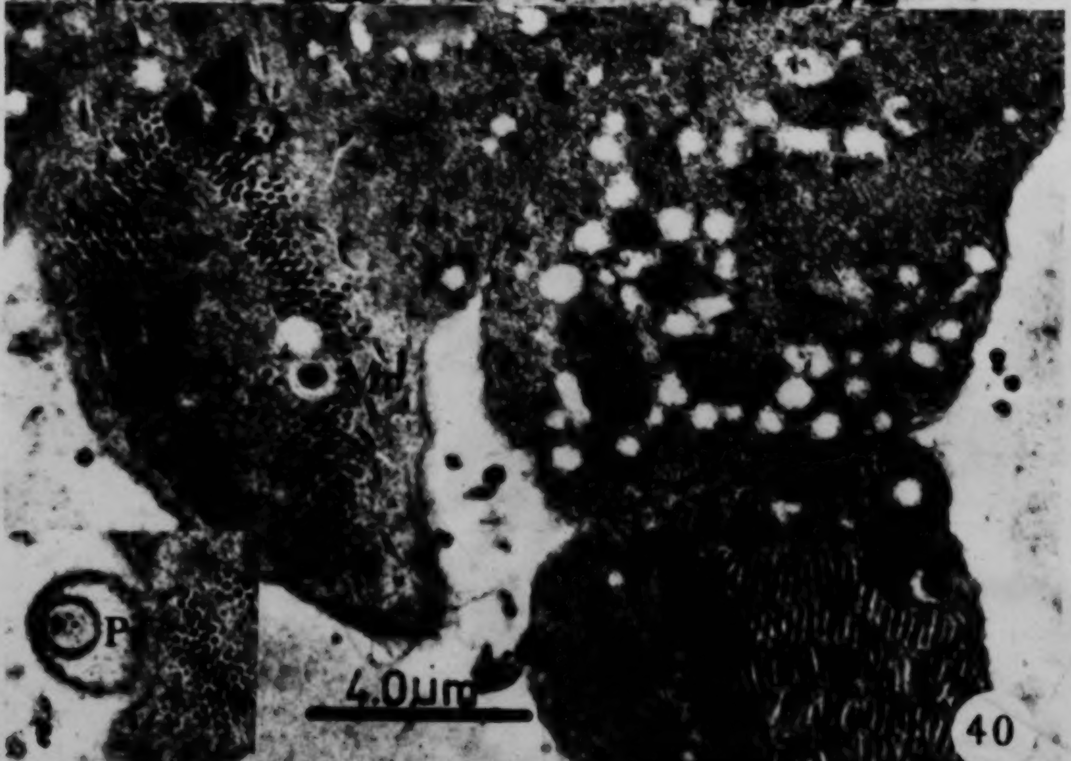
C

Figures 39 - 40. Electron micrographs of Sarcocystis from moose, Tye II cyst wall and cystozoites.

Figure 39. Detail of primary cyst wall showing digitiform projections, gaps (arrows) and fibrillar bundles (fi) in granular zone (Gz).

Figure 40. Cystozoites, note orderly arrangement of micronemes (Mn). Also present are rhoptries (Rh), mitochondria (M) and a micropore (Mp). Inset. Transverse section through conoid region. Note polar ring (Pr) with rhoptrie necks inside and the 22 subpellicular microtubules.


pcw
scw
Gz
um
39


P
4.0um
40

Outside the pcw was a well developed secondary cyst wall (scw) (Fig. 38) composed of disrupted host tissue. The Type II scw was characterized by three distinct layers:

a) The layer nearest the pcw was a loose aggregation of debris such as host cell mitochondria, myofibrils and loose granular material.

b) The second layer was a uniform (39 um), moderately electron dense zone with no apparent structure.

c) The outer layer of the scw was composed of partially disrupted myofibrils, mitochondria and host cell nuclei.

Metrocytes were encountered frequently in the Type II cysts (Fig. 37). They were delimited by a typical pellicular complex and were observed in several stages of differentiation.

The structural features of the cystozoites of the Type II cysts were similar to those of the Type I cysts with the exception of the size, number and organization of the rhoptries and micronemes. In cystozoites from the Type II cysts there were fewer rhoptries, the micronemes were in a more highly ordered arrangement and they were larger in diameter (60 nm) (Fig. 40, 40a).

CHAPTER IV DISCUSSION

Each aspect of the results will be discussed separately (i.e. prepatent period, endogenous stages and cyst structure). Aspects of the Sarcocystis life cycle, as it occurs naturally, are dealt with speculatively in a separate section. A section discussing taxonomy and nomenclature within the genus Sarcocystis follows. The concluding segment presents an overview of the study and outlines areas in which further research might be concentrated.

DEFINITIVE HOST, PREPATENT PERIOD

Based on knowledge of the most likely and consistent moose predators and the life cycle completions of other cervid - Sarcocystis life cycles (white-tailed deer - dog, Drouin 1976; mule deer - dog/coyote, Hudkins-Vivion et al. 1976) it was a strong possibility that dogs would prove to be a suitable definitive host for moose Sarcocystis. Thus, the emphasis of life cycle completion attempted in this study was, at the outset, placed in this canid as the definitive host.

Failure of the attempt to infect a coyote should be viewed with caution. Studies by Fayer and Johnson (1975) and Hudkins-Vivion et al. (1976) have indicated that sarcocysts from domestic oxen (Bos taurus) and deer (Odocoileus hemionus) are infective to both dogs and coyotes. The experimental coyote in this study was fed a small amount of sarcocyst-infected meat and none was available to be fed to a dog which would act as a positive control. Thus, the viability of the sample was unknown. It is also possible that a light infection may have gone undetected.

Cats were used to test the possibility that the life cycle would involve felids. The cats also served as controls against the possibility of the presence of a second, not readily detectable, species of Sarcocystis.

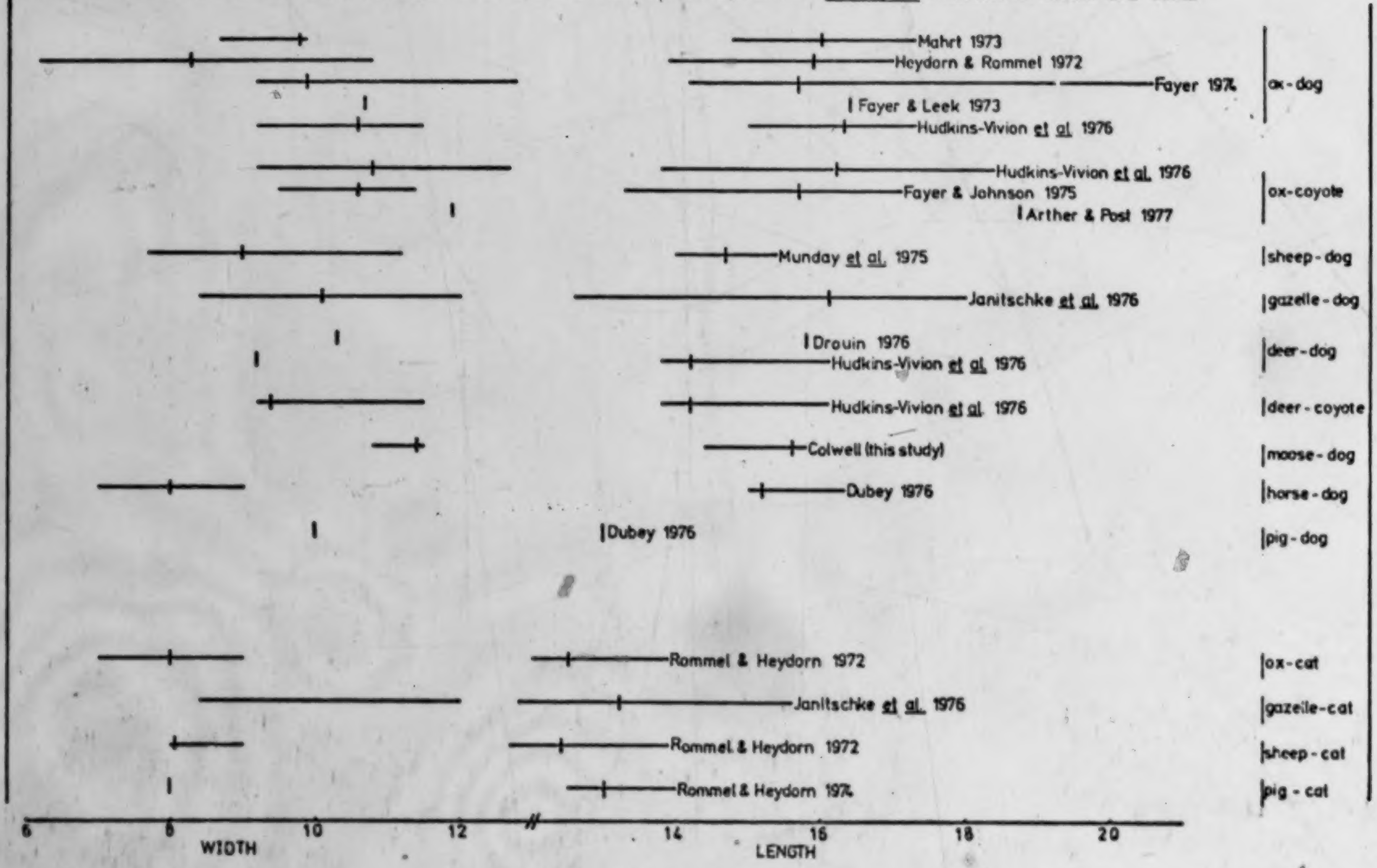
The prepatent period reported in this study for dogs infected with moose origin Sarcocystis is within the ranges reported for other life cycles which involve canids (Heydorn and Rommel 1971, Fayer 1974, Munday et al. 1975, Fayer and Johnson 1975, Hudkins-Vivion et al. 1976). The maximum range reported is 9-22 days (Fayer, 1974) although this is an extreme example with special circumstances.

The overlap reported for the prepatent period of various species of Sarcocystis (range between 9-15 days) is in contrast to the wide range known to exist between species of the genera Eimeria and Isospora (sensu strictu) (Levine, 1973 and Kheysin 1972). A partial explanation may be found in the lack of asexual stages in the definitive host of the Sarcocystis life cycle. Whereas the isosporans and eimerians have a variable number of asexual generations, both between species (Levine 1973) and in some cases between strains of the same species (MacDougald and Jeffers 1976) and perhaps extraintestinal wanderings (particularly in the case of Isospora), the different species of Sarcocystis all undergo exactly the same sequence of developmental events (i.e. gametogony and sporogony). The limited variability of the prepatent period in Sarcocystis can be explained on the basis of asynchronous penetration of intestinal cells by cystozoites, slight asynchrony of development, and delays involved with release of the oocysts or sporocysts from the subepithelial tissues.

The structural features of sporocysts recovered from the dogs fed infected moose meat and described in this study do not differ in any notable way from those described from a variety of other definitive hosts (Rommel et al. 1972, Heydorn & Rommel 1972, Mehlhorn & Scholtyseck 1974, Munday et al. 1975, Fayer 1974, Fayer & Johnson 1975, Ruiz & Frenkel 1976, Dubey 1976). The sporocysts of ungulate origin Sarcocystis which have been described to date, differ primarily in the nature of the sporocyst residuum. The sporocyst residua described in this study were always compact, appeared to be membrane bound and were usually spherical. In other species the residua vary from compact to highly dispersed. The taxonomic importance of the residuum is questionable as it has been observed that in most coccidia the sporocyst residua change both in size and in organization over time (Kheysin 1972, Duszynski et al. 1977). It has been suggested that the residuum contains nutritive material which is utilized by the sporozoites while they await their release into a suitable host environment (Kheysin, op. cit.). At present there is only circumstantial evidence to support the above contention, and it may be that the residuum is nothing more than unused material remaining after sporozoite formation. Such residual masses are common remnants of merozoite formation in the asexual portion of the classical coccidian life cycle.

A summary of published sporocyst dimensions for the known carnivore-ungulate Sarcocystis spp. is presented in Figure 41. The measurements are grouped according to intermediate and definitive hosts. In only one case (Hudkins-Vivion et al. 1976) has it been shown that sporocysts produced by infection of a single definitive host species with material from two different intermediate host species are statistically distinct (Fig. 41). Further comparisons are not possible without access to raw data or unless authors include standard deviations or standard errors with their results (Sokal & Rohlf 1970). It remains, however, that sporocyst dimensions alone are of no value in identifying

Figure 41. Sporocyst dimensions (means & ranges) from reports of natural and experimental Sarcocystis infections in canids and felids.



the origin of an infection and they are of minimal value in species descriptions. Several authors (Kheysin 1947, Becker et al. 1955, Duszynski 1971, Samoil & Samuel 1977) have noted that the dimensions of _Eimeria_ oocysts change during the course of the patent period. It may be instructive to look for similar changes in sporocyst size during the _Sarcocystis_ patent period. The cautionary remarks of Samoil & Samuel (1977) with regard to placing taxonomic emphasis on a potentially variable feature would further bear on the case of _Sarcocystis_ if variability were demonstrated.

ENDOGENOUS STAGES: GAMONTS AND OOCYSTS

This study found that developing gamonts and oocysts of _Sarcocystis_ were present throughout the length of the small intestine but that heavy concentrations were present in the anterior half. Oocysts and other stages were generally found in the luminal half of villi. Munday _et al_. (1975) reported a similar distribution of _Sarcocystis_ developmental stages in dogs fed sarcocyst infected sheep muscles. Fayer (1974), Heydorn & Rommel (1972) & Mehlhorn and Scholtyseck (1974) reported that developing oocysts were concentrated in the posterior third of the small intestine of dogs fed bovine tissues (Fayer 1974, Heydorn & Rommel 1972) and cats fed bovine tissues (Mehlhorn & Scholtyseck 1974). Fayer (_op. cit_.) also noted that oocysts were found in the middle third of the villi. The question is thus raised as to whether the cystozoites of the different _Sarcocystis_ spp. exhibit a site specificity (albeit rather broad) or whether the various observed distributions are the result of other factors (age and condition of cystozoites, size and consistency of food bolus, etc.). Long and Millard (1976) have demonstrated that sporozoites of three species of chicken coccidia are capable of migrating to their normal intestinal or cecal site of development when inoculated into a "down stream" site or intraperitonally. Studies on site specificity in Sarcocystis might be of value in distinguishing between species which infect the same definitive host.

The developmental stages observed in the dog intestine were similar to those reported for other ungulate-carnivore Sarcocystis (Fayer 1974, Munday et al. 1975, Mehlhorn & Scholtyseck 1974) and also similar to those reported from rodent-carnivore life cycles (Zaman & Colley 1975, Ruiz & Frenkel 1976). Few microgametocytes and early acrogametes were observed; similar difficulties were noted by Fayer (1974) and Munday et al. (1975). The problem of finding microgametocytes and early macrogametes may have been due to the relatively small size of these stages (Zamen & Colley 1975), the relatively low numbers and the presumably short developmental period. Multiple feeding of the experimental hosts was used as a possible solution to the above problems, but was not successful.

Oocysts were present in intestinal tissue of those dogs whose infections were 7 days duration or longer. Fayer (1974) also reported the appearance of oocysts in intestinal tissue of dogs 7 days after per os infection with an ox Sarcocystis. Munday et al. (1975), however, reported that oocysts were present in dog intestine 4 days after receiving sarcocyst infected sheep muscle. The latter authors reported a 3-day lag period between appearance of occysts and onset of sporogonic development. This is an interesting observation but perhaps indicates that Munday et al. (1975) were mistaken in their identification of the oocysts.

The sporogonic events outlined in this study were similar to those reported by Fayer (1974) and Munday *et al*. (1975). Sporulation in *Sarcocystis* oocysts, as seen in tissue sections, followed the same sequence of events as has been observed in fresh, unfixed oocysts of *Isospora* spp. (Mahrt 1968, Shah 1971, Lepp & Todd 1976). Similarities in this particular portion of the life cycle may be cited as further evidence of close affinities between the genera, *Sarcocystis* and *Isospora*. Further discussion of sporogony is included in the following section.

Electron microscope observations made on the endogenous stages in the dog intestine were limited as a result of two particular conditions:

(1) Low intensities of infection in those experiments (III & IV) designed for elucidation of early developmental stages, and

(2) the nature of the oocyst and sporocyst walls which prevented the proper penetraiton of fixatives and embedding media in many cases. Some features of macrogametes, developing and sporulating oocysts were clear enough for close examination.

Vetterling *et al*. (1973) reported that oocyst wall formation in *Sarcocystis*, grown in cultured cells, was similar to that known for eimerians (reviewed by Scholtyseck *et al*. 1971). Zaman and Colley (1975) described a similar process for *S. orientalis* in the Malaysian Reticulated Python (*Python reticulatus*). In general, oocyst wall formation involves fusion of the WFBI into a single layer beneath the sporont membrane, thereby forming the outer oocyst wall. In *Eimeria* & *Isospora* a second group of WFB (WFBII) coalesce forming several membranes which ultimately fuse to form the inner oocyst wall. Inner wall formation occurs, in Sarcocystis oocysts, in a fashion which strongly resembles that known for the eimerians (Vetterling et al. 1973) despite the absence of WFBII in Sarcocystis. Vetterling et al. (op. cit) found this fact remarkable given the marked differences in oocyst wall thickness between Eimeria spp. (approx. 3 um) and Sarcocystis sp. (see Table 4). It was noted in this study and by Mehlhorn and Scholtyseck (op. cit.) that in many cases the oocyst wall was absent around sporulating sporocysts, having apparently ruptured.

Table 4 compares the dimensions of several macrogamete and oocyst features from a variety of coccidians. The features compared are the WFB, the oocyst wall, the sporocyst wall and the intravacuolar tubular inclusions which will be discussed in a subsequent paragraph. Several species of Eimeria are compared with Isospora, Toxoplasma, and the known Sarcocystis spp. With regard to the WFB, there is a distinct size difference between those reported from Sarcocystis spp. and those from coccidians. A marked difference in WFB size was noted in comparison of this study with studies by Vetterling et al. (1973) and Zaman and Colley (1975). Further study of the moose Sarcocystis, including growth in cultured cells is necessary to verify the large difference in size of WFB. Variation in oocyst wall width between the several species of Sarcocystis is also quite marked. Sporocyst wall widths from this study are similar to those from Mehlhorn and Scholtyseck (1974).

Studies on both eimerians and Sarcocystis have indicated that the sporont, within the oocyst, has two membranes surrounding it (Scholtyseck et al. 1971, Mehlhorn and Scholtyseck 1974, Vetterling et al. 1973). This study indicated the presence of two membranes around

Table 4 - Dimensions of Macrogamete & Oocyst Structures for Several Representative Coccidians

STRUCTURE					ORGANISM	AUTHOR
WFB I	WFB II	OOCYST WALL	SPOROCYST WALL	TUBULAR INCLUSIONS		
.31-.55 nm	-	890-1300 A	890-1550 A	670 A	*S.* sp (I)	this study
610-820 nm	-	60-70 nm	-	-	*S.* sp	Vetterling *et al*. 1973
-	-	.25 um	1600 A	-	*S. tenella*	Mehlhorn & Scholtyseck 1974
90-650 nm	-	0.8 um	-	-	*S. orientalis*	Zaman & Colley 1975
.3 um	.6 um	-	-	-	*I. felis*	Pelster, 1973
.5	1.2	-	-	-	*I. felis*	
.3	.9	-	-	-	*T. gondii*	
.7-1.4 um	1.2-1.8 um	-	-	-	*E. tenella*	Doens-Juteau & Senaud 1973
.35 um	.7 um	-	-	480 A	*E. ferrisi*	Chobotar *et al*. 1975
.5-1.4 um	.5-1.4 um	-	-	790-970 A	*E. marina*	Scholtyseck *et al*. 1971
.3 um	1.0 um	-	-	700-900 A	*E. falciformis*	

the sporont, at least one of which was incomplete. Freeze-fracture studies of Eimeria neischulzi showed that sporont to be limited by a multimembrane complex (Dubremetz _et_ _al_. 1975). The conflict between thin-sectioned observations and the freeze-fracture observations remains to be resolved.

The coccidian sporocysts wall is generally considered to be composed of two layers (Speer _et_ _al_. 1973). There is evidence which indicates that the layers are formed in a manner similar to that known for the oocyst wall (Appendix II). Mehlhorn & Scholtyseck (1974), in a study of _Sarcocystis_ development in cats, found sporocyst structure resembled that known for _Isospora_ (Speer _et_ _al_. _op_. _cit_.). The present study confirmed a bilayered sporocyst wall structure for _Sarcocystis_ sporocysts. The order of the thick and thin layers of the wall reported here (outer-thick; inner-thin) are opposite to what was reported by Mehlhorn & Scholtyseck (_op_. _cit_).

The intravacuolar tubules (Fig. 17) seen within the parasitophorous vacuoles of several young oocysts in this study are similar to those reported from the parasitophorous vacuole of several coccidians although other reports on _Sarcocystis_ have noted the presence of these structures. The tubules were particularly prevalent in vacuoles surrounding macrogametes. It has been suggested that the tubules are involved with a transport phenomenon between host and parasite cell. This suggestion is based on demonstrated connections between the parasite and host cell cytoplasm (Fig. 17) via the intravacuola tubules. Coccidian parasites stimulate selected aspects of host cell functions by means of some diffusable compound (Fernando _et_ _al_. 1974, Fernando and Pasternak 1977, Browning _et_ _al_. 1976). The role of intravacuolar tubules in this stimulation or in some other host-parasite interaction is not yet clear. Recent developments in isolation of intact parasitized cells (Fernando & Pasternak 1977) may lead to further understanding of the host-parasite relation and elucidation of the role of specialized structures such as intravacuolar tubules.

No cellular reaction was observed in the immediate vicinity of the parasitized host cells. This confirms the observations made by other authors (Fayer 1974, Munday *et al*. 1975, Zaman & Colley 1976, Mehlhorn & Scholtyseck 1974) who have studied the endogenous stages of the carnivore portions of the *Sarcocystis* life cycle. *Sarcocystis* probably elicits little host immunity to reinfection. Fayer (1974), Hudkins-Vivion *et al*. (1976), and Ruiz & Frenkel (1976) were able to reinfect both dogs, coyotes, and cats, respectively, with ease although Fayer (*op*. *cit*.) noted a slight lengthening of the prepatent period in challenge infections. A recent paper by Rose & Hesketh (1976) indicates that the sexual stages of the highly immunogenic chicken coccidian *Eimeria maxima* are of little importance to the establishment of immunity in the infected host. The above authors (Rose & Hesketh *op*. *cit*.) have demonstrated that the asexual stages, particularly those involved in pathogenesis, are the most immunogenic. That information would lead one to expect that Sarcocystis would not be a highly immunogenic organism.

In order to better understand any intracellular parasite it is necessary to know which cells are employed as hosts for the parasite in question. *Sarcocystis* has, thus far, managed to conceal the identity of its host cell in the carnivore intestine. This study and others (Mehlhorn & Scholtyseck 1974, Fayer 1974, Munday *et al*. 1975) have indicated that mature gamonts and oocysts are generally located immediately below the epithelium but were not able to identify the type of cell parasitized. Ultrastructural data from this study showed that the parasites occupied cells adjacent to capillaries although they did not appear to be parasitizing endothelial cells. Mehlhorn and Scholtyseck (1974) suggested that the parasitized cells were actually sunken epithelial cells. Ruiz & Frenkel (1976) and Munday *et al*. (1975) report the presence of some early gamonts near the basal lamina of epithelial cells. These results lend some support to the suggestion made by Mehlhorn and Scholtyseck (*op*. *cit*.) but further study of the matter is indicated.

SPOROGONY

Few coccidian species are known which have oocysts that sporulate within the tissue of their host. Kheysin (1972) cites only four examples among classical coccidians of which two are probably part of a *Sarcocystis* life cycle. *In situ* sporogony is an interesting phenomenon in light of what is known about the conditions which are necessary for sporulation in free oocysts.

In his discussion of sporogony, Kheysin (1972) indicated that lack of O_2 and temperatures in excess of 30°C severely limit development within oocysts. Sporulation times for exogenously sporulating coccidia range from 2-6 days (at 20°C) with a decrease in time observed as the temperature increases to 30°C. Those species which have extremely thick oocyst walls are known to have much longer sporulation times (e.g. *Eimeria Leukarti*: 21-22 days at 20°C); presumably a result of the difficulty of O_2 diffusion into the sporont.

Wagenback and Burns (1969) have measured O_2 utilization in sporulating oocysts and have correlated different levels of uptake with the occurence of various events in the sporogonic sequence. Aerobic metabolism would be a necessary requirement in sporulating oocysts because of the limited nutrient reserves in the closed system. In addition, sufficient nutrient reserves must be maintained within the newly formed sporozoites for maintenance during storage, and to provide energy for penetration of host cells after excystation.

If it is considered that the Sarcocystis oocysts are fully formed by 4 days p.i. and sporocysts are not present in the feces until day 11-15 post-infection then it appears that sporogony in Sarcocystis takes approximately 6-10 days. This extended period of sporogonic development may be the result of a relatively low O_2 availability in the subepithelial environment. Close proximity to capillary blood flow would at least ensure a constant supply of O_2. The thin, often ruptured oocyst wall may be an adaptation which allows the differentiating cells greater access to available oxygen. It must also be presumed that those species which utilize mammalian or bird definitive hosts have adapted to the high internal temperatures of the host body. The reported snake Sarcocystis life cycles (Rzepczyk 1974, Zaman & Colley 1975) do not include data on prepatent periods or the length of time required for in situ sporogony. Until this becomes available we will have no clue as to the effect of the variable reptilian body temperature on sporulation.

Knowledge of the structural details of sporogony is at present limited because of technical problems involved with penetration of oocyst and sporocyst walls by fixatives and embedding materials.

Studies by Canning and Sinden (1973) and Canning & Morgan (1976) on various sporozoans (Eimeria tenella, Hepatozoan domerquei, Plasmodium berghei) have given support to a hypothesis that meiosis in the Sporozoa in post-zygotic. The meiosis is thought to occur as a single division within the sporont. No structural evidence is yet available to confirm this hypothesis although in Sarcocystis, as in other coccidia, the first nuclear division is a prominent and unique occurrence.

Preliminary freeze-fracture study of the sporogony in oocysts of Eimeria nieschulzi (Appendix II) has shown that the nuclear divisions which occur during sporogony have some features in common with the well studied nuclear divisions in coccidian schizonts (Dubremetz 1973). The nuclear membrane persists throughout the division, and the nucleus appears to be pulled apart. Present at opposite ends of the dividing nuclei are spindle-like centrocones with attached tubules running into both nucleo- and cytoplasm. The freeze-fracture technique allows few observations of non-membrane bound structures and thus an examination of intranuclear processes is unlikely. As a result no confirmation of meiotic hypothesis is possible via a freeze-fracture study.

The sequence of events following the first nuclear division has been described in two previous studies (Fayer 1974, and Munday et al. 1975). Fayer (op. cit.) clearly illustrates a sequence of cytokinesis, 2nd and 3rd nuclear divisions and sporozoite differentiation following the first division. Munday et al. are less clear in their illustrations and report that the 2nd nuclear division and the first cytokinesis occur together. The data from this study are identical to that reported by Fayer (op. cit.).

CYST STRUCTURE

Gross and histological examination of moose muscles for the presence of sarcocysts revealed that the distribution of muscle cysts was extensive and apparently not restricted to any particular body region (see Appendix I). Kaliner (1974), in his examination of African artiodactyls occasionally found individuals in which the distribution of sarcocysts was limited to certain muscle groups. Sampling error may be considered an explanation of these findings. Fayer and Johnson (1974) have shown a rather general distribution of cysts in experimentally infected calves. Because the inocula were so large as to cause death of the calves it must be considered that the distribution of parasites may be an experimentally induced artifact.

The histomorphology of moose sarcocysts seen in paraffin sections is not unusual. Similar cyst morphologies can be seen in many descriptions (Rzepczyk 1974, Kaliner 1974, Howells et al. 1975, Ruiz & Frenkel 1976).

The difficulty in distinguishing between the two cyst types in paraffin sections made a study of the distribution of each cyst type impossible. Was the limitation of Type II cysts to tongue, esophagus and diaphragm an observational artifact? Further detailed examination of this portion of the life cycle is a necessary step in further understanding of this parasite.

Both Type I and Type II cysts have fine structural features which are common to other described Sarcocystis cysts. In this section I will discuss the present state of knowledge with respect to the origins and functions of these common features. Developmental studies by several authors (Senaud 1967, Mehlhorn et al. 1975a, b) have indicated that the primary cyst wall (pcw) is a highly modified and hypertrophied parasitophorous vacuole membrane. It is now generally assumed that the parasitophorous vacuole membrane is of host origin. It may be an altered plasmalemma carried in by the penetrating parasite or it may be a specially formed membrane produced de novo by the host cell. The factors which stimulate thickening and elaboration of the parasitophorous vacuole membrane to produce the pcw are not known but are undoubtedly under the direction of the parasite. The morphological changes in the vacuole membrane which result in the pcw accompany the increase in numbers ofzoites within the cyst. An interesting parallel is seen in the structure of the parasitophorous vacuole membrane which surrounds the giant first generation schizonts of Eimeria bovis. Sheffield and Hammond (1966) presented photos of the schizont parasitophorous vacuole membrane which bears a striking resemblance to the developing pcw seen in the work of Mehlhorn et al. (1975a, b).

There is very little knowledge of the functions performed by the pcw. Acid phosphatase, alkaline phosphatase and ATPase have all been located in the pcw by ultracytochemistry (Mehlhorn et al. 1974, Mehlhorn 1975) but little can be inferred from the presence of these ubiquitous enzymes. It would seem plausible that the pcw is a selective barrier involved with the transport of nutrient and other compounds into the cyst and perhaps with transport of wastes out of the cyst. Presence of the above mentioned enzymes would be consistent with a transport function.

The presence of micropore-like structures and vesicle-like invaginations in the pcw of many cysts thus far described is suggestive of a phagocytic type of activity, but there is no direct evidence to support this contention. The presence of micropore-like structures in the pcw is quite remarkable given the presently accepted view that the pcw is derived from a membrane which is of host cell origin (Scholtyseck et al. 1974). It is of course not known to what extent the parasite contributes to the ultimate make-up of the pcw.

During the maturation of the cysts studied by Senaud (1967) and Mehlhorn et al. (1975a, b, c) the pcw became either highly folded or developed a dense covering of microvillar-like extensions. The function of these structures would seem to be to increase the surface area of the cyst. A similar function could be ascribed to the extensions of the pcw seen in both Type I and Type II cysts.

It has been shown by Mehlhorn and Scholtyseck (1973) that the state of development of the secondary cyst wall (scw) is at least partially a function of the age of the cyst. However, it is becoming evident, as more species of Sarcocystis are examined ultrastructurally, that the development of the scw is a characteristic of the species. At present no explanations are available for the presence of a well developed scw around some species and its absence from around others.

The granular zone immediately below the pcw and the septa which are associated with the granular zone are complete enigmas. Both the origins and the functions are unknown and there has been little speculation on the subject. It is possible that the materials composing both the septa and the granular zone are residual products remaining from the metrocyte divisions. In many cases the cystozoites are enveloped by the granular material; perhaps the material is nutritive or is a medium used to transport material from the cyst wall to the developing metrocytes and cystozoites.

The presence of a great deal of rough endoplasmic reticulum and numerous free ribosomes in the cytoplasm of the cystozoites is indicative of a high degree of synthetic activity. Organization of the r.e.r. in some instances suggests that it is involved in production of the rhoptries and micronemes. These two structures are thought to contain enzymes which play a role in penetration of the host cell membrane. Recent work by Jensen and Edgar (1976) and Kilejian (1976) indicate that the contents of the rhoptries play a role in increasing the host membrane lability which thus allows entry of the parasite. Mehlhorn *et al*. (1975) described the development of the rhoptries and micronemes in *Sarcocystis tenella*; however, none of the features they described corresponded with what I have described here as being involved in the development of the two structures mentioned. A more thorough examination of the process is necessary to clarify the interpretations made in this study with respect to the work of Mehlhorn *et al*. (*op*. *cit*.).

ASPECTS OF THE NATURAL LIFE CYCLE

The establishment of dogs as definitive hosts for a species of Sarcocystis infecting moose led to some speculation on how the life cycle might be occuring in the field. By virtue of their size and pack hunting tactics wolves (*Canis lupus*) are the only canid species present in the moose range which are capable of being consistent and effective predators of these large cervids (Mech 1970).

Frenzel (1974) in his review of North American wolf scat analysis studies indicated that cervids are the major food source for wolves. Moose generally represent a small percentage (1-37%) of the total cervid kill (Frenzel *op*. *cit*., Mech *op*. *cit*.). Areas such as Isle Royale where virtually no other cervids are present are exceptions. The general consensus on wolf food habits is that it is not necessarily abundance of a prey species which dictates its frequency in the wolf diet, but the availability or accessibility of the prey which seems to be the importance factor (Frenzel _op_. _cit_., Mech _op_. _cit_., Pimlot 1959, Cowan 1947, Peterson 1955). Thus, even when moose densities are high, they often do not represent a large proportion of the wolf diet. No specific data is available on the importance of moose in the diet of wolves in Alberta, but it is known that cervids constituted 52% of the dietary items recovered from the gastro-intestinal tracts of wolves killed as a result of the 1974-75 wolf control program (Gunson _et_ _al_. 1976).

Reviews of wolf predation on moose (Frenzel 1974, Mech 1970) indicate that during the summer and fall a high proportion of the animals killed are calves. Data collected by Mahrt (pers. comm.) indicates that mature cysts were present in young of the year moose collected during the fall at Elk Island National Park. If this is true in other localities then calves may make an important contribution to the epizootiology of _Sarcocystis_. The oldest moose are the next most utilized age class in terms of wolf predation. "Old" moose (estimated age 7-9 yr.) examined during the course of this study were all heavily infected and their contribution to the epizootiology of _Sarcocystis_ would be significant.

Fayer (1974) demonstrated that dogs previously infected with beef _Sarcocystis_ were not immune to reinfection. This fact, which requires more detailed study, allows one to infer that each time a wolf feeds on an infected moose it will shed infective sporocysts. A wolf pack would thus have a tremendous potential for "seeding" their habitat with

Sarcocystis sporocysts. However, given the low moose content in wolf diet it is difficult to explain the high prevalence of *Sarcocystis* infected moose in Alberta if wolves are the sole definitive host.

The contribution of coyotes and red foxes to the epizootiology of Sarcosporidiosis is strongly suggested by the situation which exists in Cypress Hills Provincial Park and Elk Island Park. In both areas moose (and other cervids) are infected with *Sarcocystis* in the absence of wolves. The prevalence is low (30%) in moose in the Cypress Hills Provincial Park (Barrett - pers. comm.) but in the Elk Island National Park population the prevalence of infection is similar to that of the moose in other areas of the province (Mahrt - pers. comm.) Recent reports (see Gier and also Ables in Fox 1975) indicate that both coyotes and red foxes will feed extensively on carrion when it is available. Both of these canids must be considered as potential definitive hosts; the one experimental failure not withstanding.

The longevity of *Sarcocystis* in beef carcasses (Fayer 1975a) suggests that infection of definitive hosts through consumption of carrion is a viable possibility. Field conditions differ from those of slaughtered beef thus the viability of sarcocysts in a moose carcass in the field may not approach that reported by Fayer (*op*. *cit*.).

The possibility that the overlapping cervid populations in Alberta (i.e. *Alces alces*, *Cervus canadensis*, *Rangifer tarandus*, *Odocoileus hemionus*, *O*. *virginianus*) might be sharing one or more species of *Sarcocystis* must also be considered if we are searching for an explanation of the high prevalences in all species of cervids. Known heavy usage of cervids by carnivores (e.g. Gunson 1976) and scavengers (Gier 1975) would make the epizootiology of a "shared species or species complex" most interesting. Sporocysts shed by infected canid definitive hosts would be infective to any of the cervid species which would in turn harbor cysts infective to the various canids.

Studies on intermediate host specificity of Sarcocystis (Table 5) and studies on host specificity of classical coccidia (Vetterling 1976, Box 1975, Doran 1953, Samoil 1976, Marquardt 1973 for an overview) appear to contradict the hypothesis of a shared species made in the preceeding paragraph. The basic tenet of Vetterling (1976) and Samoil (1976) is that the ability of a coccidian species to infect a new host species is directly related to the phylogenetic distance between the "normal" host species and the new host species. Marquardt's (1973) review of host specificity indicates that in general the classical coccidians (Eimeria, Isospora) have strict specificity, a species of coccidian usually being restricted to a single host genus and in many cases to a single host species.

There are some striking exceptions to the above generalizations. Wacha and Christiansen (1974, 1976) have noted that the host range of Eimeria spp. of snakes and turtles is broad. Haberkorn (1971) reported that E. contorta was infective to both mice and multimammate rats. Hendricks (1977) reports infecting primates (of several families), canids, felids and other carnivores with a single species of Isospora.

The examination of host specificity in Sarcocystis is still at an elementary level, but a trend indicating a strict intermediate host specificity, and a somewhat more plastic definitive host specificity is emerging (see Table 5 for intermediate hosts and Fayer et al. (1976) for definitive hosts). The situation with Sarcocystis can be contrasted with that of Toxoplasma gondii which is known to have an extremely low host specificity.

Table 5 - A Summary of Recent Attempts at Cross-Transmission of *Sarcocystis* spp. to Various Intermediate Hosts.

Source	Definitive Host	Experimental Inter. Host	Success	Author(s)
Mule deer	coyote	ox	neg.	Hudkins & Kistner 1977
		sheep	neg.	
ox	dog	pig	neg.	Fayer *et al*. 1976
		monkey	neg.	
		rat	neg.	
		mouse	neg.	
		rabbit	neg.	
		sheep	neg.	
sheep	dog	ox	neg.	Munday 1976a
sheep	cat	ox	neg.	
ox	dog	pig	neg.	Munday 1976b
ox	dog	sheep	neg.	Fayer & Johnson 1975 Gestrich *et al*. 1974
ox	coyote	sheep	neg.	Fayer & Johnson 1975
?	coyote	sheep	neg.	

According to Landau (1976) the eimerians represent the highest order of specialization among the "coccidiomorphs"; possessing a strict one host life cycle. "Cyst forming coddidiomorphs" are a less specialized group representing the ancestral condition. Toxoplasma would thus be considered the least specialized member of the "cyst forming" group. The obligate two host life cycle known for sarcocysts would seem to place it between the eimerians and Toxoplasma according to Landau's (1974) phylogeny. It might then be reasonable to infer that the host specificities would follow a similar trend; Toxoplasma having a broad specificity, the eimerians having a narrow specificity and Sarcocystis possessing an intermediate condition. A report by Drouin (1976) of a deer-dog-duck transmission is very provocative with respect to the specificity problem, but the report is in need of verification.

If phylogenetic relatedness of the hosts is a limiting factor in the ability of a coccidian to infect more than one species then it would be important to examine relationships between various intermediate hosts of Sarcocystis. As seen in Table 4 most of the cross-transmission attempts with ungulate Sarcocystis have involved passages between members of the Bovinae & Caprinae or Cervidae & Bovidae. In the following paragraph I will briefly examine the present state of affairs concerning phylogenetic relations between bovids, caprids, and cervids.

Flerov (1952) indicated that the separation of the Cervinae (Cervus, Alces, etc.) from Neocervinae (Odocoileus, Rangifer) took place in the lower Miocene. The two subfamilies developed and flourished separately (Cervinae, Old World; Neocervinae, North America) until the Pleistocene invation of North America by members of the Cervinae.

The bovids and cervids appear to have diverged from a common stem in the late Eocene or early Oligocene (Romer 1966, Flerov 1952). The basal stock from which these groups were derived is clear, and there is little data on which to base relationships between the major divisions; most of which were already present as distinct groups in the Old World Pliocene (Romer *op. cit.*). In a recent study of the comparative gross cerebral anatomy of the Artiodactyla, Giffin (1974) suggests that the present bovina and caprina arose from stems which were separated in the very early Miocene. This indicates a long standing separation of the two groups.

Wurster and Benirschke (1968) point out that basically similar karyotypes do not necessarily imply a close phylogenetic relationship whereas dissimilar karyotypes are considered indicative of a lack of relatedness. The karyotypes of the cervids under consideration show a great deal of similarity (2n=70) (Hsu & Benirschke 1967).

Karyotypes of bovids and caprids reveal distinct differences (*Bos taurus* 2n=60, *Ovis aries* 2n=54) (Hsu & Benirschke, 1967) between these subfamilies. This indicates a substantial phylogenetic distance between the Bovinae and the Caprinae. The phylogenetic distance between the Bovidae and the Cervidae is also illustrated by this karyotyping data.

A review of Levine & Ivens' (1970) compilation of ungulate coccidia indicates that with the exception of a few disputed cases bovids and caprids do not share any species of *Eimeria*. Thus muskox (*Ovibos moschatus*) whose phylogenetic affinities appear to be between the *Bovinae* and the Caprinae has been found to have a number of distinctly caprid eimerians (Duszynski *et al.*, 1977). No sharing is noted between bovids and cervids.

It is my suggestion that the five cervid genera in Alberta (and for that matter elsewhere in North America) share at least one species of Sarcocystis. Testing of this hypothesis would involve ultrastructural examination of sarcocyst morphology and completion of the life cycle of both the intermediate and definitive hosts. A statistical analysis of sporocyst dimensions as done by Hudkins-Vivion et al. (1967) may give further indications of similarities or differences although the value of this approach is limited (see earlier Discussion). Final testing of the hypothesis would require cross-transmission studies. It is possible that the results of such a study might have implications with regard to the relationships between various bovid and cervid genera. Host specificities of parasites have in a number of cases been instrumental in the clarification of relationships between the hosts (e.g. Hopkins, 1957).

Of particular importance to the epizootiology of Sarcocystis is the hardiness of the sporocysts once they are released into the external environment. Eimerian and isosporan oocysts are known to last for varying periods of time depending on their microenvironment, but under appropriate laboratory conditions (stored at $4^{\circ}C$ in K_2Cro_7) remain viable and infective for several years (Kheysin 1972). No published studies deal with survival of Sarcocystis sporocysts, however, indications from the attempts made to store sporocysts recovered during this study are that the sporocysts are less hardy than eimerian and isosporan oocysts maintained under the same conditions.

It is important to the understanding of the parasite epizootiology to know more about the acquisition of infective stages by intermediate hosts. Reports on the food habits of moose (Peterson 1955, 1974, Stevens 1974, LeResche and Davis 1973) indicate heavy usage of woody browse with use of available forbs and grasses in the spring and fall and moderate to heavy utilization of grasses and aquatic vegetation (if available) in the summer. Based on the food habit data and on knowledge of sporocyst hardiness the most likely time for moose to acquire viable sporocysts is during the summer with spring and fall being also likely times for acquisition. Developmental studies by Fayer and Johnson (1974) indicate approximately 30-40 days are required for development of cysts in cattle. Given a similar development time for moose _Sarcocystis_ data from Mahrt (pers. comm.) noting cysts in young of the year moose collected in December and from Hudkins & Kistner (1977) noting cysts in fawns collected in late spring would support the above contention concerning time of acquisition. Samuel (1972) postulated that eggs of the cestode _Taenia krabbei_ might be concentrated in aquatic habitats by spring runoffs. _Taenia krabbei_ has a moose-wolf cycle resembling that postulated for _Sarcocystis_ and the concentration of infective stages might operate similarly in both cases.

During this study it was found that _Sarcocystis_ sporocysts did not retain viability, in storage, for as long a period as did oocysts of various other coccidians kept under identical conditions. _Sarcocystis_ sporocysts were not viable after 6 months whereas oocysts of _Isospora canis_ and _Eimeria nieschulzi_ were viable after almost 3 years in storage. Possibly sporocysts of _Sarcocystis_ do not contain enough nutrient to maintain sporozoites for extended periods. If, in fact, the sporocyst residuum contains nutritive material which is available to the sporozoites then there is likely no shortage of nutrients. Alternatively the short storage time of _Sarcocystis_ sporocysts may be a function of the lack of protection from environmental vicissitudes which results from the lack of the oocyst wall. Observations made on sporocysts after six months storage support this contention. The sporocysts' were swollen and contents were disrupted.

In a summary of recent research Dubey (1976) points out that the pathogenicity, in the intermediate host, is variable among the presently known species of Sarcocystis. The development of some species proceeds with no clinical signs (e.g. Sarcocystis muris, Ruiz & Frenkel 1976) whereas others produce marked clinical illness, death and abortion (e.g. Sarcocystis fusiformis Fayer and Johnson 1973, 1974). The mysterious Dalmeny disease (Corner et al. 1963), which has severe effects on a dairy herd, is now thought to have been an outbreak of sarcosporidiosis. Other outbreaks of sarcosporidiosis have since been documented (Dubey 1976, Frelier et al. 1977). Additional cases of acute sarcosporidiosis have been reported in cattle (Dubey 1976, Frelier et al. 1977) and Kistner et al. (1977) are examining the possibility of Sarcocystis involvement in a die-off of deer in Oregon.

Fayer & Johnson (1973) described the development of schizonts in endothelial cells of a wide variety of tissues from calves fed Sarcocystis sporocysts. Subsequent studies (Fayer and Johnson 1974, Johnson et al. 1975, Mahrt and Fayer 1975) have characterized the disease, pathology and serology accompanying the precystic schizogonies of Sarcocystis in calves.

To my knowledge no work has been done on the problem of intermediate host immunity to infection with Sarcocystis. The studies cited above have dealt primarily with pathogenesis with no attempts to study host response to subsequent doses of sporocysts. It would seem that on the strength of Rose & Hesketh's (1976) work that the highly pathogenic schizogenic stages would elicit a strong immune reaction to challenge infections. Ruiz & Frenkel (1976), in their study of the non-pathogenic Sarcocystis muris, reported that mice could be easily reinfected. A thorough study of the intermediate host immune response to a pathogenic species of Sarcocystis would seem to be necessary.

The potential of an outbreak of clinical sarcosporidiosis in Alberta moose is not presently known. Hudkins & Kistner (1977) have shown that a Sarcocystis present in deer (O. hemionus) is pathogenic in experimental situations. This data, correlated with field data, has been used to implicate Sarcocystis in a die-off of deer fawns. Such information makes it important that there be a more extensive examination of cervid Sarcocystis in Alberta, particularly with regard to the shared species hypothesis.

TAXONOMY

Prior to the elucidation of the Sarcocystis life cycle the taxonomy of the genus was straight forward; for each host in which cysts were found a separate species of Sarcocystis was erected (i.e. one host-one species). When more than one cyst morphology was recognized in a single host the question arose as to whether this was evidence for the presence of more than one species per intermediate host. Completion of the life cycle, the ability to establish experimental infections in both definitive and intermediate hosts and the ultrastructural study of the life cycle stages have precipitated a radical change in taxonomy within the genus.

In a summary of their work with ox (Bos taurus) Sarcocystis Mehlhorn et al. (1975d) outlined three life cycles. It had formerly been thought that only one species was present in this intermediate host. Mehlhorn et al. (op. cit.) tied each life cycle to an ultrastructurally distinct cyst morphology. These and other studies (reviewed by Dubey 1976) have demonstrated that a single host species can act as intermediate host for several species of Sarcocystis, each of which has a specific definitive host. In addition, each definitive host species can harbor infections of more than one Sarcocystis sp., each originating from a different intermediate host (Mehlhorn et al. 1975d). The cyst ultrastructure has thus assumed great importance in the designation of a species. To an equal extent the completion of the life cycle of each cyst type is important in the species description.

The ultrastructural studies of experimentally induced Sarcocystis cysts conducted by Mehlhorn et al. (1975a, b, c), Gestrich et al. (1975) and Heydorn et al. (1975a, b) have been fundamental in establishing that cyst ultrastructure is species specific. The above mentioned works have thus demonstrated those features of the cyst ultrastructure which are constant enough to be of descriptive value. These features are:

1) the general architecture of the primary cyst wall, e.g. folded, branching, invaginated,
2) the presence or absence of villus like, membranous extensions to the pcw,
3) the presence or absence of fibrils in the granular zone next to the pcw, and
4) the presence or absence of granular septa in the cyst lumen.

On the basis of data from this study and other reports of Sarcocystis, cyst ultrastructure I feel several other features can be added to those already noted:

1) presence or absence of gaps in the pcw membrane
2) presence or absence of vesicles associated with and directly beneath the pcw
3) presence or absence of digitiform projections of the pcw
4) the nature of the secondary cyst wall (e.g. is the integrity of surrounding muscle tissue retained or is the tissue organization disrupted and stratified).

Table 6 compares the ultrastructural features of those ungulate *Sarcocystis* species which have been studied. A clear distinction between each of the species present in one host species is possible using the noted features. The Sarcocystis species from different hosts are also distinguishable from one another.

From Table 6 it can be seen that the structure of *S. cruzi* and *S. fusiformis* cysts is apparently identical. *S. cruzi* with *S. hirsuta* and *S. hominis* form a complex formerly thought to be one species; *S.* fusiformis (Mehlhorn *et al*. 1975c). In North America there is apparently only one species present in ox. It is identical in most respects to *S. cruzi* but North American researchers have retained usage of the designation *S. fusiformis*.

A number of differences in cystozoite structure and cyst organization were noted between the two cyst types found in moose. Gestrich *et al*. (1975) also reported that the cystozoites (merozoites) of the three ox *Sarcocystis* species were distinct from one another and that the internal organization of the cyst was apparently characteristic for each species. Rzepczyk and Scholtyseck (1976) have also noted that

Table 6 - Comparison of the Cyst Ultrastructure of *Sarcocystis* from Ungulates

Species	Author	Host	Structural Feature							
			scw	pcw					Fibrils	Septa
					Width	Villi	Gaps	Vesicles		
S. cruzi	Mehlhorn et al. 1975	*Bos taurus*	p.d.		200-250 A	+	+	+	200 A	+
S. fusiformis	Simpson 1966	*Bos taurus*	p.d.		-	+	+	-	-	+
S. hirsuta	Gestrich *et al.* 1975	*Bos taurus*	p.d.		250 A	+	-	-	150-180 A	+
S. hominis	Mehlhorn *et al.* 1975	*Bos taurus*	p.d.		320 A	+	+	-	150-180 A	+
S. sp (Type I)	this study	*Alces alces*	p.d.	invaginations	150 A	+	-	+	-	+
S. sp (Type II)	this study	*Alces alces*	w.d.	irregular/ folded	120 A	-	+	-	200 A	+
S. tenella	Mehlhorn & Scholtyseck 1973	*Ovis aries*	w.d.	irregular/ folded	250 A	-	+	-	+	+
S. ovicanis	Mehlhorn *et al.* 1975	*Ovis aries*	w.d.	irregular/ folded	250 A	+	+	-	-	+

p.d. = poorly developed, host tissue integrity retained

w.d. = well developed, host tissue disrupted and stratified

+ = present

- = absent

in addition to distinct cyst wall structures the two species of Sarcocystis found in Rattus fuscipes have distinct cystozoites and cyst lumen arrangements. It thus appears that cystozoite structure and the arrangement of the cyst interior can be considered as good descriptive features for species of Sarcocystis. Table 7 compares cystozoite structure and cyst lumen organizaiton in the ungulate Sarcocystis species which have been studied.

On the basis of recently acquired information Heydorn *et al*. (1975) proposed a new system of nomenclature in which the specific epithet gives an indication of the two hosts involved in the life cycle by including the generic names of both intermediate and definitive hosts (e.g. *S*. *bovicanis*). Adherence to the International Code of Zoological Nomenclature has resulted in a number of exceptions to the Heydorn *et al*. (*op*. *cit*.) proposal (Levine, 1977). In those cases where the life cycle is not known it has been recommended that a provisional name be used to prevent future confusion (e.g. *Sarcocystis* sp. of the moose).

The use of the binomial specific epithet is at present an informative and descriptive form of nomenclature. It is however a distinct possibility that further research will indicate that the intermediate host specificity of *Sarcocystis* is broader than now thought. Because of the foregoing reason I feel the proposals of Heydorn *et al*. (1975) are premature.

Table 7 - Cystozoite Structure and Cyst Internal Organization of the Known Species of Ungulate *Sarcocystis*

Species	Author	Host	Features			
			Rhoptries	Micronemes	Subpellicular Microtubules	Cyst Interior
S. cruzi	Mehlhorn et al. 1975b	*Bos taurus*	14	numerous, ordered	22	zoites tightly packed, thin regular septa
S. fusiformis	Simpson 1976	*Bos taurus*	14	numerous, ordered	22	zoites tightly packed, thin regular septa
S. hirsuta	Gestrich et al. 1975	*Bos taurus*	12	numerous, ordered	22	zoites tightly packed, thin regular septa
S. hominis	Mehlhorn et al. 1975a	*Bos taurus*	6-8	few, arranged around periphery	22	zoites tightly packed, thick septa
S. sp. (Type I)	this study	*Alces alces*	8-16	loose arrangement 530 A dia.	22	zoites tightly packed, thin regular septa
S. sp. (Type II)	this study	*Alces alces*	4-7	ordered arrangement 603 A dia.	22	zoites very loosely packed, variable septa
S. tenella	Mehlhorn & Scholtyseck 1973	*Ovis aries*	11	ordered arrangement	22	zoites loosely packed
S. ovicanis	Mehlhorn *et al.* 1975c	*Ovis aries*		loose arrangement fewer	22	zoites tightly packed thin regular septa

CHAPTER V CONCLUDING DISCUSSION

The results of the life cycle completion attempts made during this study were found to be consistent, in an overall sense, with previous studies of the Sarcocystis life cycle (Mehlhorn and Scholtyseck 1974, Fayer 1974, Munday et al. 1975, Zaman and Coley 1975, Ruiz and Frenkel 1976, Dubey 1976, Levine 1977). The ultrastructural details of the various stages in the definitive host are in need of more study, both to supply additional material which can be applied to the coccidia in general (i.e. understanding the process of sporogony) and to resolve inconsistencies reported in this study.

The two cyst types described from the musculature of moose are considered to represent new species of Sarcocystis. Descriptions of each is presented as follows:

Sarcocystis sp. (Type I)

Intermediate host

Alces alces

cyst - macroscopic in tongue, esophagus and diaphragm, fusiform

- light microscopy: thin walled, the interior of mature cyst is not divided by septa.
- electron microscopy: primary cyst wall with numerous long extensions flattened against cyst, secondary cyst wall poorly developed, no fibrils present. Cystozoites tightly packed within cyst, 8-16 rhoptries and loosely aggregated micronemes.

Final host

Canis familiaris (experimental)

Sporocysts 10.8-11.5 um (11.4 ± 1.0) x 14.4-15.8 um (15.6 ± 1.2) from the feces

Prepatent period: 11-15 days

Pathogenicity: none known

Sarcocystis sp (Type II)

Intermediate host

Alces alces

cyst - macroscopic in tongue, esophagus and diaphragm, oval to spherical

- light microscopy: the wall of the mature cyst is thin and smooth, the interior of the cyst is compartmentalized by distinct septa
- electron microscopy: primary cyst wall folded into branching extensions, secondary cyst wall highly developed with three distinct strata, digitiform protrusions at p.c.w., fibrils present Cystozoites loosely packed within cyst, 4-6 rhoptries and highly ordered micronemes.

Final host

unknown

It is recommended that a thorough study of Sarcocystis in North American cervids be undertaken in an effort to test the proposal that they are sharing a common species. This study would have a fundamental value in that it would test the present concept of intermediate host specificity. Such research would also have a practical value in the realm of wildlife management as the pathogenic potential of the *Sarcocystis* species present in cervids could be assessed.

It is my opinion that the recently described *Sarcocystis hemiolatrantis* (Hudkins & Kistner 1977) should be considered *inquerenda* until a more detailed study of the cyst stages is undertaken (they are not adequately described or figured in the description) and until cross-transmission studies with other intermediate hosts (cervids and other ungulates) can be completed.

PLATE ABBREVIATIONS

A	amylopectin
cap	capillary
Co	conoid
Cy	cystozoite
Gz	granular zone
H	host tissue
Hn	host cell nucleus
ivt	intravacuolar tubules
M	mitochondria
me	metrocyte
Mn	microneme
Mp	micropore
N	nucleus
nm	nuclear membrane
Np	nuclear pores
pcw	primary cyst wall
Pr	polar ring
pv	parasitophorous vacuole
rbc	red blood cells
rer	rough endoplasmic reticulum
Rh	rhoptries
scw	secondary cyst wall
se	septa
wfb	wall forming bodies

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Appendix I - Summary of Gross and Histological Examinations of Moose for the Presence of *Sarcocystis* during Sept. 1975 - Dec. 1976

	SEX	LOCALITY	HISTOLOGICAL EXAMINATION					MACROSCOPIC EXAMINATION
			HEART	TONGUE	ESOPHAG.	DIAPHR.	SKEL. MUS.	
MS 1	M.	White Court	+	+	/	/	/	-
MS 2	M.	?	/	+	/	/	/	-
MS 3	F.	Lesser Slave	/	+	/	/	/	+
MS 4	M.	Edson	+	+	+	+	/	+
MS 5	F.	Edson	+	+	+	+	+	+
MS 6	F.	Edson	/	+	/	/	/	-
MS 7	F.	Grande Cache	/	+	/	/	/	-
MS 8	F.	Wolf Lake	+	+	+	/	/	+
MS 9	M.	Grande Cache	+	+	+	+	/	+
MS10	F.	Grande Cache	+	+	+	+	/	+
MS11	F.	Edson	+	+	+	+	+	+
MS12	M.	Freeman Lake	/	+	/	/	/	+
MS13	M.	Swan Hills	/	+	/	/	/	-
MS14	M.	Fort Assin.	/	+	/	/	/	+
MS15	M.	Folley Lake	/	+	/	/	/	-
MS16	M.	Goose Lake	/	+	/	/	/	-
MS17	M.	Mud Creek	/	+	/	/	/	+
MS18	M.	Judy Creek	/	+	/	/	/	+
MS19	M.	Roche Lake	/	+	/	/	/	-
MS20	M.	Erikson Lake	/	+	/	/	/	+
MS21	M.	Keenawa Ridge	/	+	/	/	/	+
MS22	M.	Swan Hills	/	+	/	/	/	-
MS23	M.	Swan Hills	/	-	/	/	/	+
MS24	F.	Fort Assin.	/	-	/	/	/	-
MS25	M.	Swan Hills	/	-	/	/	/	-
MS26	M.	Swan Hills	/	-	/	/	/	-
MS27	M.	Caribou Lake	/	+	/	/	/	+
MS28	M.	Folley Lake	/	+	/	/	/	+
MS29	M.	Time Creek	/	+	/	/	/	+
MS30	M.	?	-	-	/	/	/	-
MS31	M.	?	-	+	/	/	/	-
MS32	M.	?	-	-	/	/	/	-
MS33	M.	Roche Lake	-	-	/	/	/	-
MS34	M.	?	/	/	/	/	/	-
MS35	M.	?	+	+	/	/	/	-
MS36	F.	Edson	+	+	+	+	/	+*
MS37	M.	Athabaska	/	+	+	/	/	+*
MS38	M.	Athabasca	/	+	+	/	/	+
MS39	M.	Athabasca	/	-	-	/	/	-
MS40	M.	Roche Lake	/	+	+	/	/	+
	31M-9F.		9/13	31/39	10/11	6/6	2/2	21/40

* Two types of macrocysts seen
+ Sarcocysts present
- Sarcocysts not present
/ Tissue not available for examination

Appendix II - Freeze-fracture observations on sporulating *Eimeria nieschulzi* oocysts.

INTRODUCTION

Detailed examination of sporogony in coccidian oocysts has been severely limited by the presence, in most genera, of highly impervious oocyst and/or, sporocyst walls. The majority of studies on sporogony have been restricted to light microscope observations on fresh material (see Hammond, in Hammond and Long, 1973). This is particularly true in the genera Eimeria and Isospora. Sarcocystis is a notable exception in that it is impossible to make limited light and electron microscope observations on stages fixed and embedded _in situ_ in the host gut.

Because chemical fixation and subsequent epoxy embedding is not required the freeze-fracture technique provides a unique possibility for expanding our view of the details of sporogony. The most important limitation of this technique is that it is useful for studying only those structures which are membrane bound.

Preliminary freeze-fracture work on oocysts of _Eimeria neischulzi_ was undertaken in order to examine the feasibility of the technique. Details of the structure of unsporulated oocysts has been reported by Dubremetz _et al_. (1975). Some of the data obtained from freeze-fracture of sporulating oocysts is presented here, as an Appendix, because of its relevance to the discussion of sporogony in _Sarcocystis_.

METHODS AND MATERIALS

Two coccidia free rats received _per os_ inoculations of 50,000 sporulated oocysts of _Eimeria neischulzi_. Seven days post infection, the entire fecal output of both rats was collected and the fresh unsporulated oocysts were recovered, cleaned and concentrated according to standard procedures. Oocysts recovered from feces at 8:00 a.m. were considered unsporulated (0 hours). Those oocysts to be examined immediately were washed in distilled water, pelleted by centrifugation and resuspended in 30% glycerol. The remaining oocysts were suspended in 2.5% KCr_2O_7, placed into large beakers through which air was continuously bubbled and kept at room temperature. Aliquots of oocysts were removed from the beakers at 12, 24, 48, and 72 hours, cleaned and suspended in 30% glycerol.

Oocysts placed in glycerol were allowed to equilibrate for at least one hour prior to use. Small drops of the oocyst slurry were placed on gold specimen discs and quenched in liquid Freon 22. The specimens were fractured and carbon-platinum replicas were prepared at -100°C in a Balzer's freeze-etch apparatus. Replicas were cleaned in chromic acid and bleach and were observed in a Phillips 200 or 201 transmission electron microscope.

RESULTS AND DISCUSSION

The sporont and sporoblasts, at all stages of development, contained those organelles and inclusions reported in the unsporulated sporont by Dubremetz _et_ _al_. (1975) and Birch-Andersen (1976). An increase in the amount of endoplasmic reticulum was noted in the undivided sporont 12 hours after aereation was begun.

At present most of the observations on sporulating oocysts, deal with the nuclear divisions. As noted in light microscope studies on sporogony and in ultrastructural studies of other life cycle stages a nuclear division begins with elongation of the nucleus. Figure 1 shows a portion of an elongating nucleus. Note that the nuclear membrane is present and will remain throughout the entire process of division.

Dubremetz (1973) has described a unique structure associated with the nucleus during division in schizonts of Eimeria necatrix. The centrocone is a centriole-like structure present in the nuclear membrane which is attached to microtubules with origins in both nucleo- and cytoplasm. A centrocone is present at each end of the dividing nucleus and it is thought that they are involved in the "pulling apart" of the nucleus. The box in Figure 1 centres on a structural feature in the nuclear membrane which resembles a centrocone. If it is a centrocone it establishes that the nuclear division in sporogony are of the same character as those known for other live cycle stages.

Figures 2 and 3 show a partially complete and a completed nuclear division, respectively, as seen in sporoblasts. A thin "neck" connects the two daughter nuclei in Figure 2. The overall paucity of nuclear pores in these dividing nuclei is in striking contrast to the condition seen in unsporulated sporonts (Dubremetz et al. 1975).

Observations made on sporoblasts prior to formation of sporozoites have indicated that the sporocyst wall has not been completed. The sporoblasts are delimited by three membranes (Fig. 3). This resembles the unsporulated sporont (Dubremetz et al. 1975) and also the developing oocyst within the host (Scholtyseck et al. 1971). Close examination of the sporoblasts revealed the presence of numerous granules aligned along the inner membrane (Fig. 4). Occasionally a granule will appear to be fusing with the inner membrane (Fig. 4) in a manner similar to that known for oocyst wall formation (Scholtyseck et al. 1971).

Interpretation of freeze-fracture replicas is often difficult without the aid of accompanying thin-sectioned material. This difficulty has limited the interpretation of events in the process of sporogony. Birch-Andersen _et al_. (1976) have described a technique involving sectioning of frozen oocysts with subsequent fixation and embedding for obtaining electronmicrographs of structures within coccidian oocysts. While the concept is worth developing the technique described by the above authors is very likely to introduce severe artifact. Oocysts frozen in liquid Freon and stored in liquid nitrogen (-180°C) were warmed to -30°C for sectioning prior to fixation. This amount of warming would produce large ice crystals and disrupt cellular organization. A modified freeze-substitution technique involving sectioning of oocysts at -100°C and fixation in cold acetone-osmium tetroxide (-80°C) would be less likely to produce artifact. Further study of the events in sporogony using a combination of the two techniques will no doubt result in a clear picture of the process.

Figure 1. Electronmicrograph of freeze-fractured _Eimeria_ _nieschulzi_ oocysts. Portion of elongating sporont nucleus (N). Note abundant endoplasmic reticulum and what may be a centrocone (box).

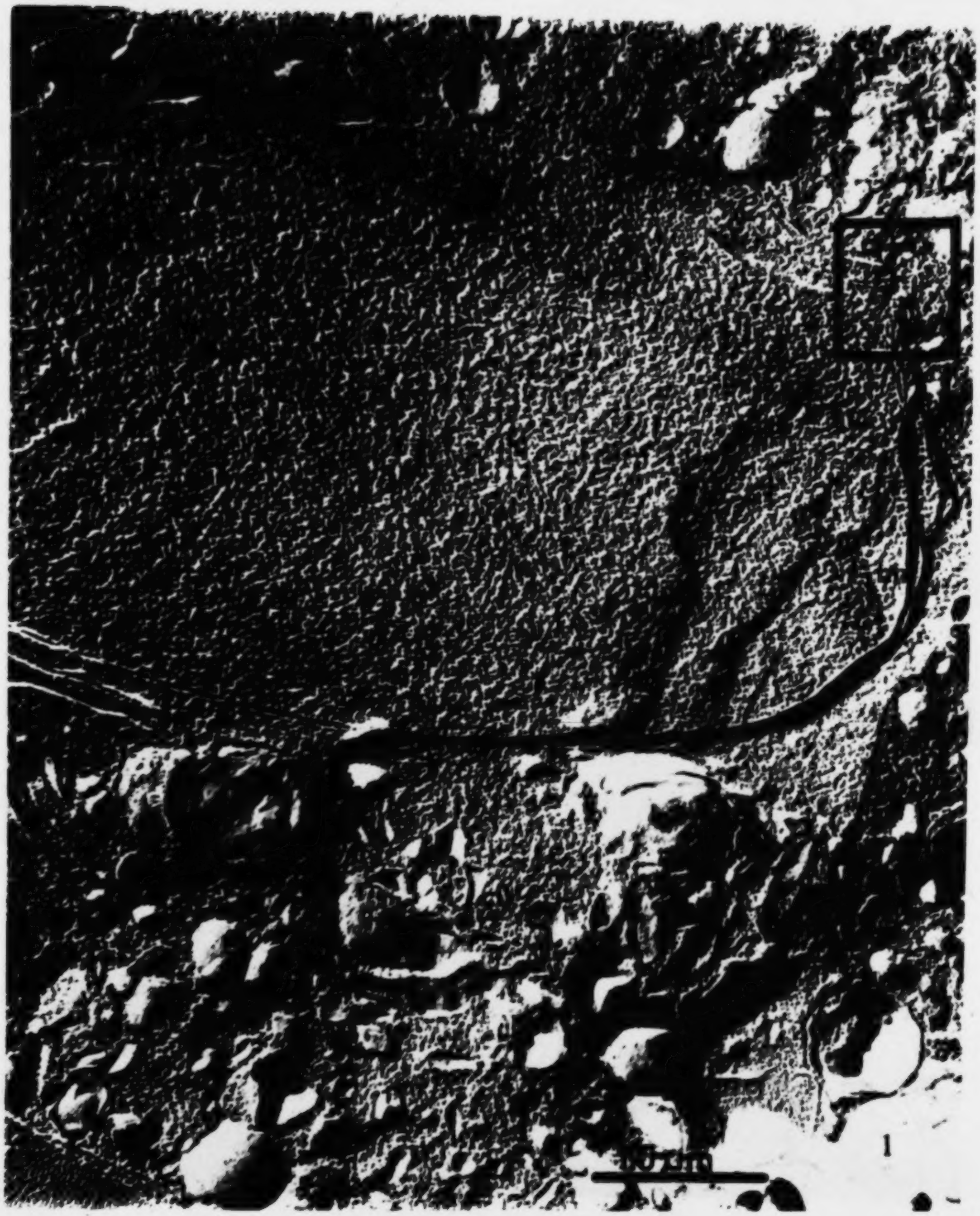

500nm
1

Figure 2. Electronmicrograph of freeze-fractured *Eimeria nieschulzi* oocysts. Dividing nucleus in sporoblast. Only a thin stalk joins the portions of the dividing nucleus.

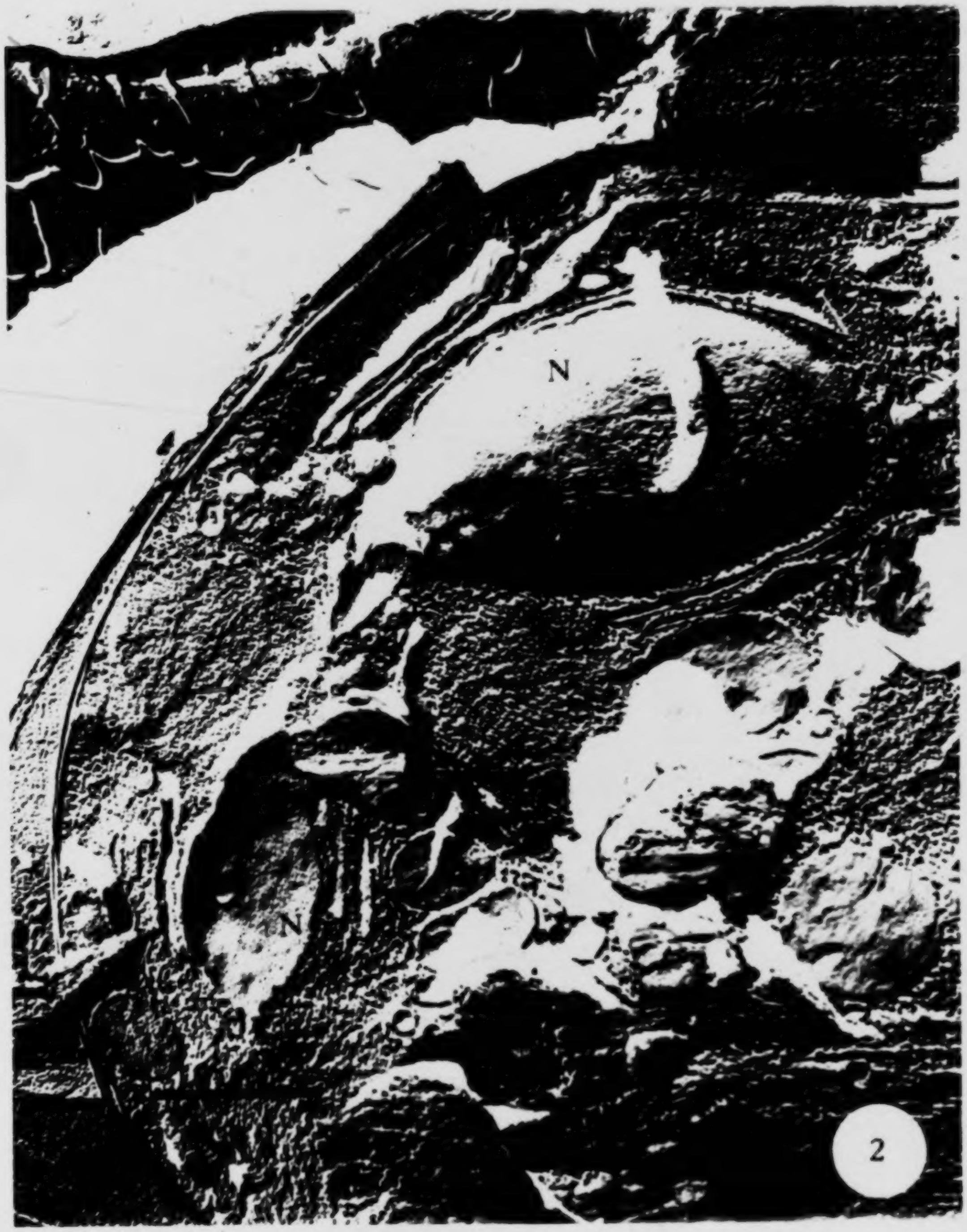

N
N
2

Figure 3. Electronmicrograph of freeze-fractured Eimeria nieschulzi oocysts. Nuclear division in the sporoblast is complete but sporozoite differentiation is not yet apparent.

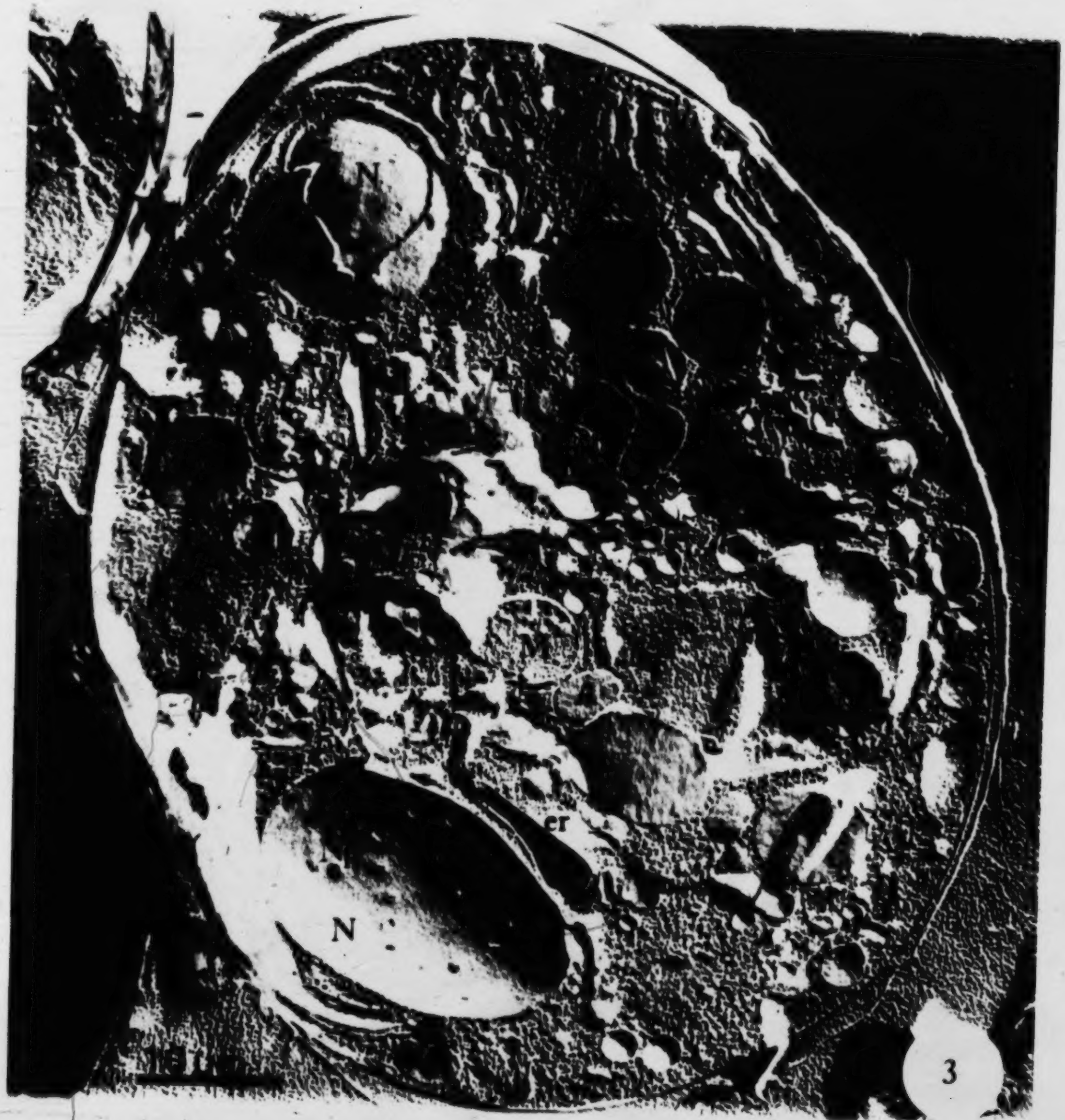
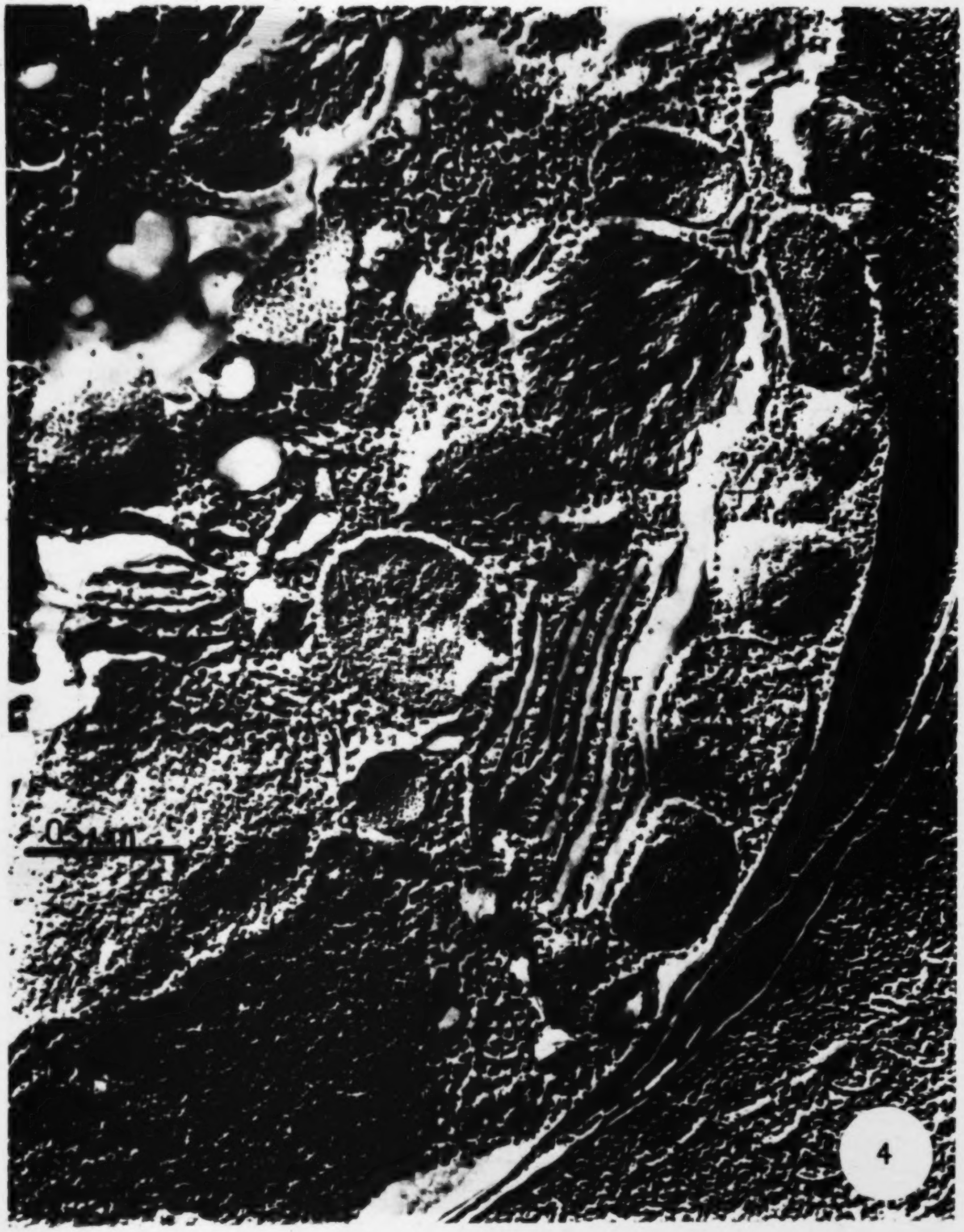

N
er
N
3

Figure 4. Electronmicrograph of freeze-fractured Eimeria nieschulzi oocysts. Detail of developing sporocyst wall. Note the 3 limiting membranes, the vesicles aligned along the inner membrane and one vesicle apparently fusing with the inner membrane (top right).


0.5 µm
4